February 1980 Vol. 56 No. 1

British Journal of
Venereal Diseases

2

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

R N THIN *(Editor)*
J D ORIEL
B EVANS
A McMILLAN *(Abstracts)*
(Assistant Editors)
C B S SCHOFIELD
(*President, MSSVD*)

J BARROW
(*Hon. Sec., MSSVD*)
J WALLIN
G CAUSSE
J-M KNOX
A E WILKINSON

I PHILLIPS
G CSONKA
E STOLZ
J THIVOLET
L M DRUSIN

EDITOR
British Medical Journal
SHUNA JUDGE
(Technical Editor)

This *Journal,* founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A E Wilkinson, 22 Kingsend, Ruislip, Middlesex HA4 7DA. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals,* available from the Publishing Manager, *British Medical Journal,* BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; 55: 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS. *Venereal Diseases.* 3rd ed. London: Baillière and Tindall, 1975.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 214-22.

PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £23.00 in the United Kingdom and Republic of Ireland and US$55.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US$55.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal,* 1172 Commonwealth Avenue, Boston, Mass. 02134. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases,* BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1980 by the *British Journal of Venereal Diseases.* This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.

CUK-H06333-3-P25895

British Journal of Venereal Diseases

Edited for the Medical Society for the Study of Venereal Diseases

EDITOR R N Thin

ASSISTANT EDITORS B Evans, A McMillan

EDITORIAL COMMITTEE C B S Schofield, J Barrow, M W Adler, G Causse, G Csonka, L M Drusin, J M Knox, G D Morrison, I Phillips, E Stolz, J Wallin, A E Wilkinson, Editor of the *British Medical Journal*

TECHNICAL EDITOR Shuna Judge

②

VOLUME 56, 1980

P 25895

BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, LONDON WC1H 9JR

Contents

February 1980 Vol 56 No 1

The International Union against the Venereal Diseases and Treponematoses
R D CATTERALL (Gt Britain) *page* 1

Characterisation of *Neisseria gonorrhoeae* from women with simultaneous infections at two sites
ROBERT C NOBLE (USA) *page* 3

Homosexuality and venereal disease in the United Kingdom
BRITISH CO-OPERATIVE CLINICAL GROUP (Gt Britain) *page* 6

Recent observations on the serology of syphilis
A LUGER, B SCHMIDT, I SPENDLINGWIMMER, AND F HORN (Austria) *page* 12

Non-specific positive test results to syphilis in dermatological diseases
M GIBOWSKI AND E NEUMANN (Poland) *page* 17

Antitreponemal IgE in early syphilis
J D BOS, F HAMERLINCK, AND R H CORMANE (the Netherlands) *page* 20

Auxotypes and antimicrobial suceptibilities of *Neisseria gonorrhoeae* in black and white patients
ROBERT C NOBLE AND B R MILLER (USA) *page* 26

Relationship of the interval between infections and the similarity of gonococcal strains in recurrent gonorrhoea
ROBERT C NOBLE, A P ORANJE, M F MICHEL, AND E STOLZ (USA and the Netherlands) *page* 31

An unusual case of gonococcal arthritis
K M RENNEY (USA) *page* 35

Chlamydial infection of the cervix in contacts of men with nongonococcal urethritis
I A TAIT, E REES, D HOBSON, R E BYNG, AND M C K TWEEDIE (Gt Britain) *page* 37

Urine culture for the detection of *Trichomonas vaginalis* in men
A WILSON AND J P ACKERS (Gt Britain) *page* 46

Herpesvirus hominis type 2 infection in Ibadan
D MONTEFIORE, A O SOGBETUN, AND C N ANONG (Nigeria) *page* 49

Sexually transmitted diseases in clinic patients in Lagos
V O ROTIMI AND A O SOMORIN (Nigeria) *page* 54

Correspondence *page* 57

Notices *page* 57

Book review *page* 58

Abstracts *page* 59

April 1980 Vol 56 No 2

Some aspects of venereal diseases in Greenland
ELLIS FROM (Denmark) *page* 65

Immunoglobulin-bearing lymphoid cells in primary syphilis
J D BOS, F HAMERLINCK, AND R H CORMANE (the Netherlands) *page* 69

T lymphoid cells in primary syphilis
J D BOS, F HAMERLINCK, AND R H CORMANE (the Netherlands) *page* 74

Further evaluation of the microhaemagglutination test to determine treponemal antibodies in CSF
S KASATIYA AND A BIRRY (Canada) *page* 77

Assessment of the Till-U-Test GC slide
W H DONALD (Gt Britain) *page* 81

Delay and inhibition of growth of *Neisseria gonorrhoeae* in vitro
WILLIAM G WESTBROOK III (Philippines) *page* 83

Prolonged use of the Greenland method of treatment of gonorrhoea
A J EVANS, G D MORRISON, AND D J PRICE (Gt Britain) *page* 88

Value and feasibility of screening women attending STD clinics for cervical chlamydial infections
SHIRLEY J RICHMOND, I D PAUL, AND P K TAYLOR (Gt Britain) *page* 92

Colonisation of pregnant and puerperal women and neonates with *Chlamydia trachomatis*
P-A MÅRDH, I HELIN, S BOBECK, J LAURIN, AND T NILSSON (Sweden) *page* 96

Treatment of nongonococcal urethritis with trimethoprim-sulphadiazine and with placebo
J PAAVONEN, M KOUSA, P A SAIKKU, E VARTIAINEN, L KANERVA, AND A LASSUS (Finland) *page* 101

Carcinoma of the bladder presenting as gonococcal cystitis
A L BLACKWELL AND J BARROW (Gt Britain) *page* 105

Vaginal pH and microflora related to yeast infections and treatment
S M DRAKE, B A EVANS, AND A GERKEN (Gt Britain) *page* 107

Proctitis and herpes simplex virus in homosexual men
D GOLDMEIER (Gt Britain) *page* 111

First African Regional Conference on Sexually Transmitted Diseases
R D CATTERALL (Gt Britain) *page* 115

Correspondence *page* 117

Book review *page* 118

Abstracts *page* 119

June 1980 Vol 56 No 3

Influence of testicular fluid infected with *Treponema pallidum* on intradermal lesions
T J FITZGERALD AND R C JOHNSON *page* 125

Sulfhydryl oxidation using procedures and experimental conditions commonly used for *Treponema pallidum*
T J FITZGERALD, R C JOHNSON, AND E T WOLFF *page* 129

Utrastructure of vascular and connective tissue changes in primary syphilis
TERESA WRZOŁKOWA AND JERZY KOZAKIEWICZ *page* 137

Identification of *Neisseria gonorrhoeae* in the routine venereological laboratory
T. RUFLI *page* 144

Prevalence of cervical gonorrhoea in women with unwanted pregnancies
B VAN DER LUGT, A C DROGENDIJK, AND J R J BANFFER *page* 148

Comparative study of bacampicillin and ampicillin in the treatment of uncomplicated gonorrhoea
PATRICIA J SPENGLER AND LARRY D EDWARDS *page* 151

Quantitative aspects of chlamydial infection of the cervix
D HOBSON, P KARAYIANNIS, RUTH E BYNG, ELISABETH REES, I ANNE TAIT, AND JEAN A DAVIES *page* 156

Epidemiology of infection by serotypes D to K of *Chlamydia trachomatis*
ERIC M C DUNLOP, SOHRAB DAROUGAR, AND JOHN D TREHARNE *page* 163

Ureaplasma urealyticum in the urethra of healthy men
J VIARENGO, F HEBRANT, AND P PIOT *page* 169

Venereal diseases in the islands of the North Pacific
R R WILLCOX *page* 173

Sexually transmitted diseases: extract from the *Annual Report of the CMO of the DHSS for the Year 1978 page* 178

Annual report of the Medical Society for the Study of Venereal Diseases *page* 182

Correspondence *page* 183

Book reviews *page* 195

Correction *page* 185

Abstracts *page* 186

August 1980 Vol 56 No 4

Editorial: Gonorrhoea—a continuing problem in the 1980s *page* 193

Sexually transmitted diseases in Nigeria
R D CATTERALL *page* 194

Pleural effusion disease agent as passenger of *Treponema pallidum* suspensions from rabbits
KNUD L FENNESTAD, LIS BRUUN, AND ERIK WEDØ *page* 198

Venereal diseases in the islands of the South Pacific
R R WILLCOX *page* 204

Immunoregulatory properties of serum from patients with different stages of syphilis
J J THOMPSON, R J MANGI, R LEE, AND J M DWYER *page* 210

Immunoglobulin-bearing polymorphonuclear leucocytes in primary syphilis
J D BOS, F HAMERLINCK, AND R H CORMANE *page* 218

Persistent serum immune complexes in syphilis
SIEGFRIED ENGEL AND WOLFGANG DIEZEL *page* 221

Detection of antigonococcal IgA in cervical secretions by indirect immunofluorescence: an evaluation as a diagnostic test
A McMILLAN, G McNEILLAGE, H YOUNG, AND S S R BAIN *page* 223

One-step staining of *Neisseria gonorrhoeae* in urethral discharge by methyl green-pyronin
N F HANNA, D TAYLOR-ROBINSON, G W CSONKA, J R W HARRIS, AND I A AL-SOWAYGH *page* 227

Induction of a cell wall variant of the gonococcus by human amylase
A MELLERSH, S HAFIZ, M G McENTEGART, AND I GEARY *page* 230

Bacterial interference of *Neisseria gonorrhoeae* by α-haemolytic streptococci
M E McBRIDE, W CHRISTOPHER DUNCAN, AND JOHN M KNOX *page* 235

Epidemiology of gonococcal vulvovaginitis among children in the tropics
K O ALAUSA AND A O OSOBA *page* 239

Penicillinase-producing gonococci in the Netherlands
H BIJKERK *page* 243

Gonorrhoea in Rotterdam caused by penicillinase-producing gonococci
K C NAYYAR, R C NOBLE, M F MICHEL, AND E STOLZ *page* 244

Antibiotic sensitivities of gonococci isolated in Rotterdam and results of treatment with cefuroxime
K C NAYYAR, M F MICHEL, AND E STOLZ *page* 249

Sensitivity of *Neisseria gonorrhoeae* to spectinomycin and thiamphenicol
K GRUNDER AND D PETZOLDT *page* 252

HR 756: a new cephalosporin in the treatment of gonorrhoea caused by ordinary and penicillinase-producing strains of *Neisseria gonorrhoeae*
V S RAJAN, E H SNG, R PANG, N J TAN, T THIRUMOORTHY, AND K L YEO *page* 255

Contact tracing in patients with genital chlamydial infection
I THELIN, A-M WENNSTRÖM, AND P-A MÅRDH *page* 259

AUGUST 1980 Vol 56 No 4 *continued*

Confidentiality in the National Health Service and in the service for sexually transmitted diseases
R D CATTERALL *page* 263

Condylomata lata of the palms: an unusual site
M EL-SAAD EL-RIFAIE *page* 267

Correspondence *page* 269

Notices *page* 270

Correction *page* 270

Book reviews *page* 271

Abstracts *page* 272

October 1980 Vol 56 No 5

Venereal diseases in the Pacific Islands: Papua New Guinea
R R WILLCOX *page* 277

Sexually transmitted diseases in Indonesia
MARWALI HARAHAR *page* 282

Mechanisms of selective toxicity of metronidazole and other nitroimidazole drugs
DAVID I EDWARDS *page* 285

Quantitative microhaemagglutination assay for *Treponema pallidum* antibodies in experimental syphilis
ROBERT R TIGHT AND ARTHUR C WHITE *page* 291

Autolymphocytotoxins in syphilis
N H L DE JONG, J A M KOEHORST, J J VAN DER SLUIS, AND A M BOER *page* 297

Concentric rings simulating tinea imbricata in secondary syphilis
P A SAROJINI, A D DHARMARATNAM, K PAVITHRAN, AND C GANGADHARAN *page* 302

Enzymatic detection of *Neisseria gonorrhoeae*
MILTON M TAKEGUCHI, HOWARD H WEETALL, DAVID K SMITH, HUGH C McDONALD, KIM A LIVSEY, CLARENCE C DETAR, AND THOMAS A CHAPEL *page* 304

Sensitivity to penicillin of gonococci in different racial groups
P RODIN, A D SETH, D M KING, AND A E WILKINSON *page* 308

Comparison of methods for the detection of penicillinase-producing *Neisseria gonorrhoeae*
E H SNG, K L YEO, V S RAJAN, AND A L LIM *page* 311

Vaginal chemoprophylaxis in the reduction of reinfection in women with gonorrhoea: clinical evaluation of the effectiveness of a vaginal contraceptive
CLIFFORD H COLE, THOMAS G LACHER, JAMES C BAILEY, AND DIANE L FAIRCLOUGH *page* 314

OCTOBER 1980 Vol 56 No 5 *continued*

Diagnosis and management of anorectal gonorrhoea in women
VALERIE A STANSFIELD *page* 319

Oropharyngeal flora and individual susceptibility to neisserial infection
H YOUNG, A B HARRIS, AND D H H ROBERTSON *page* 322

Isolation of *Neisseria lactamica* from the female genital tract
WILLIAM A TELFER BRUNTON, HUGH YOUNG, AND DONALD R K FRASER *page* 325

Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in Greenland: a seroepidemiological study
P-A MÅRDH, I LIND, E FROM, AND A-L ANDERSEN *page* 327

Tetracycline in nongonococcal urethritis: comparison of 2 g and 1 g daily for seven days
WILLIAM R BOWIE, JOHN S YU, ARCHANA FAWCETT, AND HUGH D JONES *page* 332

Subclinical pneumonia due to serotypes D-K of *Chlamydia trachomatis:* case reports of two infants
ERIC M C DUNLOP, ROGER J HARRIS, SOHRAB DAROUGAR, JOHN D TREHARNE, AND SAFWAT S AL-EGAILY *page* 337

Chlamydia trachomatis as a possible cause of peritonitis and perihepatitis in a young woman
J PAAVONEN AND V V VALTONEN *page* 341

A case of lymphogranuloma venereum of 20 years' duration: isolation of *Chlamydia trachomatis* from perianal lesions
MICHAEL DAN, HESCHI H ROTMENSCH, EMMANUEL EYLAN, ARDON RUBINSTEIN, RAFAEL GINSBERG, AND MEIR LIRON *page* 344

Correspondence *page* 347

Notices *page* 348

Correction *page* 348

Obituaries *page* 349

Abstracts *page* 350

December 1980 Vol 56 No 6

Kinetic study of serum penicillin concentrations after single doses of benzathine and benethamine penicillins in young and old people
P COLLART, M POITEVIN, A MILOVANOVIC, A HERLIN, AND J DUREL *page* 355

Penicillin concentrations in cerebrospinal fluid after different treatment regimens for syphilis
NIWAT POLNIKORN, RAWIPHAN WITOONPANICH, MALAI VORACHIT, SODSAI VEJJAJIVA, AND ATHASIT VEJJAJIVA *page* 363

Tabes dorsalis progressing to general paresis after 20 years despite routine penicillin therapy
A J H GILES *page* 368

DECEMBER 1980 Vol 56 No 6 *continued*

Immunological responses in late syphilis
R SHANNON, C G COPLEY, AND G D MORRISON *page* 372

Value of serological diagnosis in congenital syphilis: report of nine cases
M V BOROBIO, M C NOGALES, AND J C PALOMARES *page* 377

Lack of serological evidence for venereal spirochaetosis in wild Victorian rabbits and the susceptibility of laboratory rabbits to *Treponema paraluis-cuniculi*
S R GRAVES, J W EDMONDS, AND ROSAMOND C H SHEPHERD *page* 381

Susceptibility of rabbits venereally infected with *Treponema paraluis-cuniculi* to superinfections with *Treponema pallidum*
S GRAVES *page* 387

Assessment of transport and isolation methods for gonococci
E TAYLOR AND I PHILLIPS *page* 390

Gentamicin in the treatment of infections due to penicillinase-producing gonococci
V S RAJAN, N J TAN, ROGER PANG, AND E H SNG *page* 394

Rectal gonorrhoea in male homosexuals: presentation and therapy
J L FLUKER, P DEHEROGODA, D J PLATT, AND A GERKEN *page* 397

Evaluation of the Papanicolaou-stained cytological smear as a screening technique for asymptomatic gonorrhoea
GAIL I GENVERT, LEWIS M DRUSIN, JOHN F SEYBOLT, AND DAVID W BRAUN *page* 400

Prevalence of antichlamydial antibody in London blood donors
S DAROUGAR, T FORSEY, D A BREWERTON, AND K LL ROGERS *page* 404

Treatment of vulvovaginal candidal infection with miconazole-treated tampons
N A M BERGSTEIN *page* 408

Further evidence of the efficacy of co-trimoxazole in granuloma venereum
SARDARI LAL AND B R GARG *page* 412

Correspondence *page* 414

Book reviews *page* 415

Notice *page* 416

Correction *page* 416

Index *page* 417

British Journal of
Venereal Diseases

Edited for the Medical Society for the Study of Venereal Diseases

EDITOR A McMillan

ASSISTANT EDITORS B A Evans, G D Morrison

EDITORIAL COMMITTEE J Barrow, M Waugh, M W Adler, G Causse, G Csonka, L M Drusin, G D Morrison, R S Pattman I Phillips, E Stolz, D Taylor-Robinson, R N Thin, J Wallin, Editor of the *British Medical Journal*

TECHNICAL EDITOR Shuna Judge

INDEXER P D Simmons

VOLUME 58, 1982

BRITISH MEDICAL ASSOCIATION
TAVISTOCK SQUARE, LONDON WC1H 9JR

Contents

February 1982 Vol 58 No 1

Morphological destruction of cultured cells by the attachment of *Treponema pallidum*
T J FITZGERALD, L A REPESH, AND S G OAKES *page* 1

Demonstration of specific 19S(IgM) antibodies in untreated and treated syphilis: comparative studies of the 19S(IgM)-FTA test, the 19S(IgM)-TPHA test, and the solid-phase haemadsorption assay
F MÜLLER AND E-G LINDENSCHMIDT *page* 12

Alterations in T lymphocytes and T-lymphocyte subpopulations in patients with syphilis
JØRGEN R JENSEN AND ELLIS FROM *page* 18

In-vitro activity of 21 antimicrobial agents *Neisseria gonorrhoeae* in Brussels
B GORDTS, R VANHOOF, J M HUBRECHTS, R DIERICKX, H COIGNAU, AND J P BUTZLER *page* 23

Penicillinase-producing gonococcal strains in Zambia: observations on treatment failures
ATTILI V RATNAM, MOHMEDALI I PATEL, RAPHAEL C MULENGA, AND SUBHASH K HIRA *page* 29

Aetiology and management of non-specific vaginitis
I M DATTANI, A GERKEN, AND B A EVANS *page* 32

Quantitative study of *Chlamydia trachomatis* in genital infection
H MALLINSON, O P ARYA, AND A D GODDARD *page* 36

Does psychiatric illness affect the recurrence rate of genital herpes?
DAVID GOLDMEIER AND ANTHONY JOHNSON *page* 40

Incidence of HSV-1 and HSV-2 isolated from a group of patients with herpes genitalis in Sheffield
I G BARTON, G R KINGHORN, S NAJEM, L S AL-OMAR, AND C W POTTER *page* 44

Confirmation of herpes simplex virus type 2 infections in herpes-like genital lesions by a simple complement-fixation test
MINAS ARSENAKIS AND JOHN T MAY *page* 48

Aseptic meningitis and herpes simplex proctitis: a case report
W A ATIA, C S RATNATUNGA, C GREENFIELD, AND S DAWSON *page* 52

Thiamphenicol in the treatment of chancroid in men
A S LATIF *page* 54

International symposium on trichomoniasis: the Polish medical scene
R D CATTERALL *page* 56

Cell-mediated immunity in HBeAg-positive homosexuals with chronic liver disease
L VIOLA, I G BARRISON, F PARADINAS, J C COLEMAN, AND IAIN M MURRAY-LYON *page* 59

Autogenous vaccine therapy for condyloma acuminatum: a double-blind controlled study
MICHAEL D MALISON, RODNEY MORRIS, AND LAWRENCE W JONES *page* 62

Correspondence *page* 66

Obituary *page* 66

Abstracts *page* 67

April 1982 Vol 58 No 2

Editorial: statistical significances—use and misuse
A MCMILLAN *page* 71

IV Harrison Lecture 1981: the international venereological scene as viewed by Harrison and St Mary's
R R WILLCOX *page* 72

Higher medical training in venereology
C B S SCHOFIELD *page* 86

Role played by Italy in the history of syphilis
M A WAUGH *page* 92

Inhibition of *Neisseria gonorrhoeae* isolates by Martin-Lewis medium: epidemiology, susceptibility profile, and plasmid analysis
M A MILLER, P ANDERSON, J W PARKER, AND H H ROHRER *page* 96

Treatment of pharyngeal gonorrhoea due to β-lactamase-producing gonococci
MAGNUS LINDBERG, OLOF RINGERTZ, AND ERIC SANDSTRÖM *page* 101

Effects of tampon components on growth and dissemination of *Neisseria gonorrhoeae*
R J ARKO, K H WONG, K G FINLEY-PRICE AND J K RASHEED *page* 105

Rapidity and reliability of gonococcal identification by coagglutination after culture on modified New York City medium
H YOUNG AND A MCMILLAN *page* 109

Epidemiological treatment of gonorrhoea and non-specific genital infection in female sexual contacts: current practices in STD clinics in England and Wales
ELIZABETH M BELSEY *page* 113

Rosaramicin and tetracycline in the treatment of non-gonococcal urethritis: a comparison of clinical and microbiological results
J F DARNE, G L RIDGWAY, AND J D ORIEL *page* 117

Metronidazole in a single dose for the treatment of trichomoniasis: failure of a 1-g single dose
THOMAS W AUSTIN, ELIZABETH A SMITH, RIAD DARWISH, EDWARD D RALPH, AND FREDERICK L M PATTISON
page 221

Treatment of vaginal candidosis with a single 500-mg clotrimazole pessary
IAN MILSOM AND LARS FORSSMAN *page* 124

Balanoposthitis associated with *Gardnerella vaginalis* infection in men
G R KINGHORN, B M JONES, F H CHOWDHURY, AND I GEARY *page* 127

Correspondence *page* 130

Book review *page* 133

Abstracts *page* 134

June 1982 Vol 58 No 3

Effects of anaerobic and microaerophilic conditions of extraction and incubation on the survival of *Treponema pallidum* in vitro
G H W WONG, B M STEINER, AND S R GRAVES *page* 139

Interferon in rabbit sera after inoculation with *Treponema pallidum* suspensions contaminated with PED virus
K L FENNESTAD, L BRUUN, AND S HAAHR *page* 143

Evaluation of the role of the *Treponema pallidum* immobilisation test in Britain
M S SPROTT, J B SELKON, AND R H TURNER *page* 147

Syphilis d'emblée due to blood transfusion: case report
AGUS SOENDJOJO, MARIANI BOEDISANTOSO, MOCHAMAD I ILIAS, AND DIBJO RAHARDJO *page* 149

Morbidity associated with pelvic inflammatory disease
MICHAEL W ADLER, ELIZABETH H BELSEY, AND BRENDAN H O'CONNOR *page* 151

Neisseria gonorrhoeae isolated from disseminated and localised infections in pre-penicillin era: auxotypes and antibacterial drug resistances
B WESLEY CATLIN AND ALICE REYN *page* 158

Auxotypes and antibacterial resistance of gonococci with differing susceptibilities to vancomycin
A CHARLES EXNER, ELIZABETH NASH SHINNERS, PAUL J PACE, AND B WESLEY CATLIN *page* 166

Single oral dose of cefaclor for the treatment of infections with penicillinase-producing strains of *Neisseria gonorrhoeae*
THELMA E TUPASI, OFELIA V CALUBIRAN, AND CLEOTILDE A TORRES *page* 176

Treatment of uncomplicated gonorrhoea in women with talampicillin in a single oral 1·5 g dose
K C MOHANTY AND R B ROY *page* 180

Therapeutic abortion and *Chlamydia trachomatis* infection
ERIK QVIGSTAD, KJELL SKAUG, FRIDTJOF JERVE, INGER S S VIK, AND JAN C ULSTRUP *page* 182

Some factors affecting the incubation period and duration of symptoms of urethritis in men
C B S SCHOFIELD *page* 184

Ultrahistopathology of balanitis circinata
LASSE KANERVA, MERJA KOUSA, KIRSTI-MARIA NIEMI, ALLAN LASSUS, TIMO JUVAKOSKI, AND JORMA LAUHARANTA *page* 188

Rheumatoid arthritis, ankylosing spondylitis, and Reiter's syndrome occurring simultaneously: case report
H A LAVERY, T HORNER, AND S D ROBERTS *page* 196

Vaginal bleeding in granuloma inguinale: case reports
S MURUGAN, KAMALAKSHI VENKATRAM, AND P S RENGANATHAN *page* 200

Traumatic ulceration of the cervix: case report
NICHOLAS J FIUMARA AND THOMAS CAHN *page* 202

World and Latin American congresses on sexually transmitted diseases
R D CATTERALL *page* 204

Book review *page* 205

Abstracts *page* 206

August 1982 Vol 58 No 4

Scanning electron microscopy of the attachment of *Treponema pallidum* to nerve cells in vitro
LILLIAN A REPESH, T J FITZGERALD, S GEORGE OAKES, AND R S POZOS *page* 211

Electrophysiological dysfunction and cellular disruption of sensory neurones during incubation with *Treponema pallidum*
S GEORGE OAKES, LILLIAN A REPESH, R S POZOS, AND T J FITZGERALD *page* 220

Comparative in-vitro activity of selected new β-lactam antimicrobials against *Neisseria gonorrhoeae*
M Y KHAN, Y SIDDIQUI, AND R P GRUNINGER *page* 228

Treatment of uncomplicated gonorrhoea in women: comparison of rosoxacin and spectinomycin
OFELIA V CALUBIRAN, LITA B CRISOLOGO-VIZCONDE, THELMA E TUPASI, CLEOTILDE A TORRES, AND BENJAMIN M LIMSON *page* 231

Provision of a chlamydial culture service to a sexually transmitted diseases clinic
G L RIDGWAY, V MOSS, G MUMTAZ, W ATIA, A M EMMERSON, AND J D ORIEL *page* 236

Characteristics of *Haemophilus ducreyi:* a study
E H SNG, A L LIM, V S RAJAN, AND A J GOH *page* 239

Male carriage of *Gardnerella vaginalis*
S G DAWSON, C A ISON, G CSONKA, AND C S F EASMON *page* 243

Pharmacokinetics of metronidazole and its principal metabolites and their activity against *Gardnerella vaginalis*
C S F EASMON, C A ISON, C M KAYE, R M TIMEWELL, AND S G DAWSON *page* 246

Isoenzyme characterisation of *Trichomonas vaginalis*
MOHAMED ATEF SOLIMAN, J P ACKERS, AND R D CATTERALL *page* 250

Treatment of trichomoniasis with metronidazole rectal suppositories
S K PANJA *page* 257

Consulting patterns after a television programme on sexually transmitted diseases
M W ADLER *page* 259

Social factors in homosexually acquired venereal disease: comparison between Sweden and Australia
MICHAEL W ROSS *page* 263

Guillain-Barré syndrome associated with hepatitis A in a male homosexual
A DUNK, W J JENKINS, AND S SHERLOCK *page* 269

Strongyloidiasis: is it transmitted from husband to wife?
DAVID I GROVE *page* 271

IUVDT — South-east Asian and Western Pacific regional conference
R D CATTERALL *page* 273

Correspondence *page* 275

Notices *page* 276

Abstracts *page* 277

October 1982 Vol 58 No 5

Reiter haemagglutination test: a screening test for syphilis
A A AL-QUDAH AND ANN MOSTRATOS *page* 281

Comparison of the morphology, antigenicity, and pathology of Nichols and Birmingham isolates of *Treponema pallidum*
C W PENN AND J C CLAY *page* 286

Mitogenic responses of hamsters infected with *Treponema pertenue*: lack of correlation with passive transfer of resistance
J K CHAN, R F SCHELL, AND J L LE FROCK *page* 292

Depression of natural killer cell activity by syphilitic serum and immune complexes
JØRGEN RIKARD JENSEN, ANNELISE SPARRE JØRGENSEN, AND KRISTIAN THESTRUP-PEDERSEN *page* 298

Induction of phenotypically determined resistance of *Neisseria gonorrhoeae* to human serum by sera from patients with gonorrhoea
P M V MARTIN, P V PATEL, N J PARSONS, AND H SMITH *page* 302

Antibiotic sensitivity patterns of penicillinase-positive and penicillinase-negative strains of *Neisseria gonorrhoeae* isolated in Fukuoka, Japan
SHIN-ICHI YOSHIDA, SHINJI URABE, AND YASUO MIZUGUCHI *page* 305

Penicillinase-producing *Neisseria gonorrhoeae* ophthalmia neonatorum in Singapore
T THIRUMOORTHY, V S RAJAN, AND C L GOH *page* 308

Prostatitis due to penicillinase-producing *Neisseria gonorrhoeae:* case reports
C M J E VANDENBROUCKE-GRAULS, M ROZENBERG-ARSKA, C W DEN HENGST, AND J VERHOEF *page* 311

Ceftriaxone in the treatment of ordinary and penicillinase-producing strains of *Neisseria gonorrhoeae*
V S RAJAN, E H SNG, T THIRUMOORTHY, AND C L GOH *page* 314

Quinolone derivative, flumequine, as short-term treatment for gonorrhoea
H B SVINDLAND, P L SVARVA, AND J A MAELAND *page* 317

Anaerobes in men with urethritis
E A FONTAINE, D TAYLOR-ROBINSON, N F HANNA, AND E D COUFALIK *page* 321

Chlamydial screening of pregnant women in a sexually transmitted diseases clinic
B T GOH, P MORGAN-CAPNER, AND K S LIM *page* 327

Evaluation of an enzyme-linked immunosorbent assay for the detection of antibody to *Trichomonas vaginalis* in sera and vaginal secretions
D A STREET, D TAYLOR-ROBINSON, J P ACKERS, N F HANNA, AND A McMILLAN *page* 330

Carriage and transmission of group B streptococci among STD clinic patients
D H JACKSON, S M HINDER, J STRINGER, AND C S F EASMON *page* 334

Sexually transmitted diseases in India and Sri Lanka
R D CATTERALL *page* 338

STD—Sixth National Conference of the Indian Association
R D CATTERALL *page* 340

Correspondence *page* 342

Book reviews *page* 342 Notice *page* 344 Abstracts *page* 345

December 1982 Vol 58 No 6

Treponemal serology on Bali Island, Indonesia
R NEY, M F GARNER, J L BACKHOUSE, N WIRIA DUARSA, DARWATA BREGUET, AND G BREGUET *page* 351

Syphilis in pregnant women in Zambia
A V RATNAM, S N DIN, S K HIRA, G J BHAT, D S O WACHA, A RUKMINI, AND R C MULENGA *page* 355

Detection of *Neisseria gonorrhoeae* antigen by a solid-phase enzyme immunoassay
H A AARDOOM, D DE HOOP, C O A ISERIEF, M F MICHEL, AND E STOLZ *page* 359

Induction in gonococci of phenotypic resistance to killing by human serum by human genital secretions
P M V MARTIN, P V PATEL, N J PARSONS, AND H SMITH *page* 363

Chlamydial genital infection in Ibadan, Nigeria: a seroepidemiological survey
S DAROUGAR, T FORSEY, A O OSOBA, R J DINES, B ADELUSI, AND G O COKER *page* 366

Chlamydial genital infection in Addis Ababa, Ethiopia: a seroepidemiological survey
T FORSEY, S DAROUGAR, R J DINES, D J M WRIGHT, AND P S FRIEDMANN *page* 370

Chlamydial urethral infection in Teheran: a study of male patients attending an STD clinic
S DAROUGAR, BARRIE R JONES, L CORNELL, J D TREHARNE, R ST C DWYER, AND B ARAMESH *page* 374

Pathogenic microbial flora of genital ulcers in Sheffield with particular reference to herpes simplex virus and *H ducreyi*
G R KINGHORN, S HAFIZ, AND M C MCENTEGART *page* 377

Preparation and immunogenicity of vaccine Ac NFU$_1$ (S−) MRC towards the prevention of herpes genitalis
G R B SKINNER, C B J WOODMAN, C E HARTLEY, A BUCHAN, A FULLER, J DURHAM, M SYNNOTT, J C CLAY, J MELLING, C WIBLIN, AND J WILKINS *page* 381

Clinic diagnosis of anaerobic vaginosis (non-specific vaginitis): a practical guide
A BLACKWELL AND D BARLOW *page* 387

Metronidazole resistance of *Trichomonas vaginalis* as a cause of treatment failure in trichomoniasis: a case report
JAROSLAV KULDA, MILENA VOJTĚCHOVSKÁ, JAN TACHEZY, PAVOL DEMEŠ, AND EVA KUNZOVÁ *page* 394

Scissor excision of anogenital warts
JOHN M GOLLOCK, KENNETH SLATFORD, JENNIFER M HUNTER *page* 400

Screening for syphilis among homosexual men in bars and saunas in Amsterdam
L H LUMEY, JEANNETTE KOK, AND R A COUTINHO *page* 402

Ulcerative vulvitis in Reiter's syndrome: a case report
S O'N DAUNT, K E KOTOWSKI, A P O'REILLY, AND A T RICHARDSON *page* 405

Correspondence *page* 408

Book reviews *page* 410

Notice *page* 410

Index *page* 411

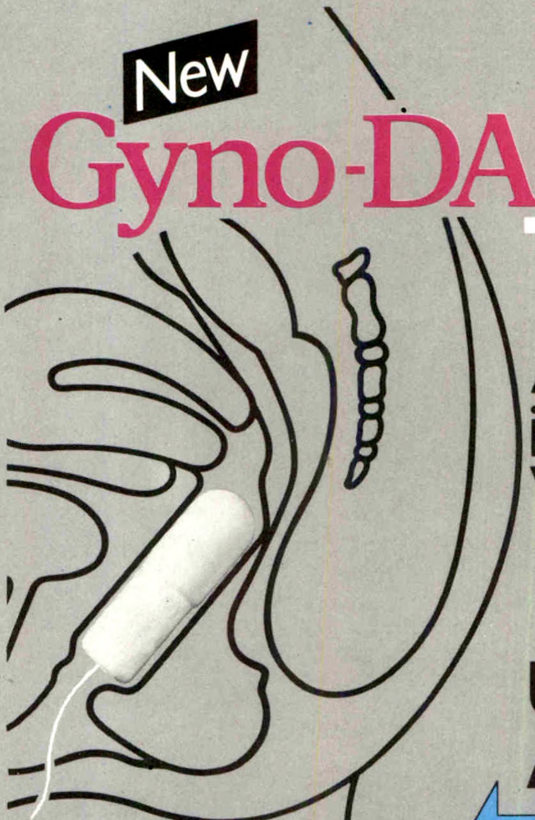
New

Gyno-DAKTARIN*

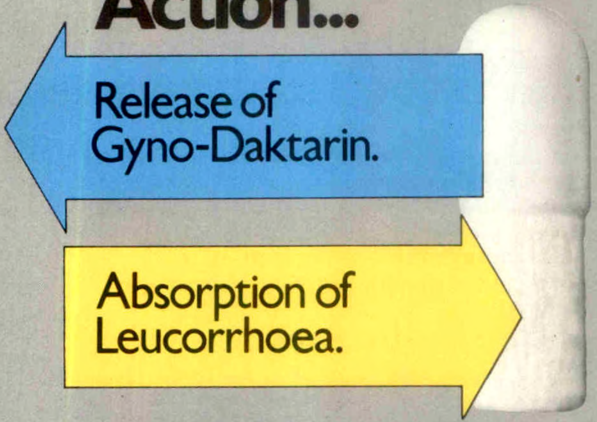
miconazole nitrate 2% w/w

Tampons

A new concept in the treatment of Vaginal Candidiasis.



Unique 2-way Action...



Comfortable, hygienic, effective.

Prescribing Information.

Presentation
Intravaginal cream: White, non-staining, water miscible cream containing miconazole nitrate 2% w/w.
Pessary: White, non-staining pessaries each containing 100mg miconazole nitrate.
Combi-pack: White, non-staining pessaries each containing 100mg miconazole nitrate plus white, non-staining, water miscible topical cream containing miconazole nitrate 2% w/w.
Tampon: tampons, each coated with 100mg miconazole nitrate.

Uses
GYNO-DAKTARIN exhibits potent anti-fungal activity and anti-bacterial activity against Gram-positive bacteria. (Strep. and Staph.) GYNO-DAKTARIN is used for the local treatment of:— Vulvo-vaginal candidiasis and superinfections due to Gram-positive bacteria, (Strep. and Staph.) Also for prophylactic treatment of the male partner and for the treatment of mycotic balanitis.

Dosage and Administration
GYNO-DAKTARIN may be used in both adults and children. Nightly, 10g of the intravaginal cream or two pessaries should be inserted high into the vagina. Disposable applicators are provided for insertion of the cream.
This procedure should be repeated for one week even though symptomatic "cure" of the pruritus and leukorrhoea will have been noted within a few days of commencing therapy.
In the case of the combi-pack, the cream should be applied topically twice daily.

GYNO-DAKTARIN tampons: one tampon should be inserted high in the vagina, twice daily (morning and evening) for five days.

Contra-indications, warnings etc.
Contra-indications: None known.
Side effects: Occasionally, irritation has been reported. Rarely, local sensitisation may occur requiring discontinuation of treatment.
Overdosage: Not applicable.

Pharmaceutical Precautions
Store in a cool place.

Legal Category
Pharmacy sale medicine.

Package Quantities
Cream: GYNO-DAKTARIN intravaginal cream is supplied in 78g tubes with disposable applicators – one week's treatment.
Pessary: GYNO-DAKTARIN pessaries are supplied in packs each containing 14 pessaries – one week's treatment.
Combi-pack GYNO-DAKTARIN combi-pack consists of 14 pessaries plus 15g topical cream.
Tampons: GYNO-DAKTARIN tampons are supplied in packs each containing 10 tampons.

Further Information
GYNO-DAKTARIN may be used with confidence in "problem vaginitis" as seen in pregnant and diabetic patients and in women using oral contraceptives.
A leaflet instructing the patient on the correct use is included in each package of GYNO-DAKTARIN.

Product Licence Numbers:

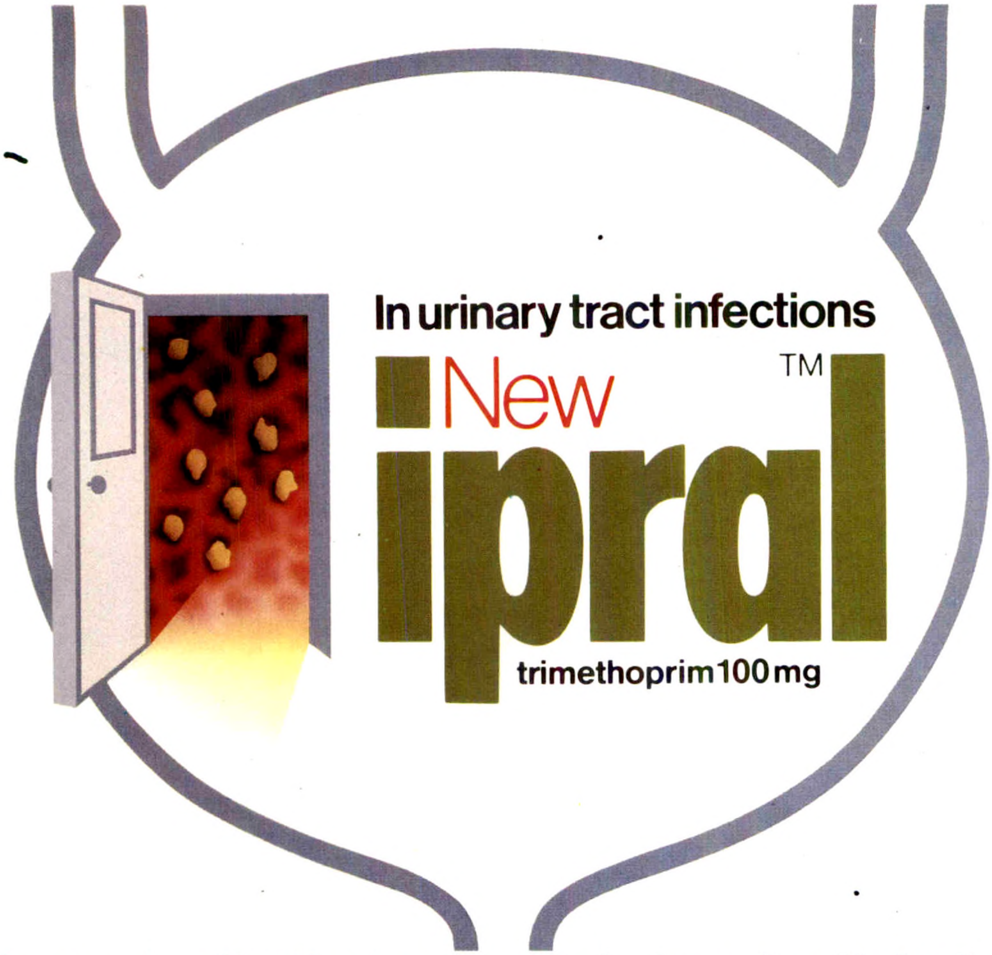
GYNO-DAKTARIN cream	PL/0242/0015	
GYNO-DAKTARIN pessaries	PL/0242/0037	
GYNO-DAKTARIN combi-pack	PL/0242/0015	PL/0242/0037
GYNO-DAKTARIN tampons	PL/0242/0056	

Basic N.H.S. cost:
Intravaginal Cream £3.96; Pessaries £3.77; Combi-Pack £4.70; Tampons £4.50.

(Correct at time of going to press)

Further information is available on request from
JANSSEN PHARMACEUTICAL LIMITED, Janssen House, Marlow, Bucks. SL7 1ET.

* Trademark



In urinary tract infections

New ipral™

trimethoprim 100 mg

...the way to effective treatment of urinary tract infections

Trimethoprim – proved effective as a single entity without the side-effects associated with sulphonamides.

"Trimethoprim alone may well prove as effective as trimethoprim-sulphamethoxazole combinations ..."[1]

96% success in general practice.[2] Half as many side-effects.

Results of 107 Courses of Treatment in 96 Domiciliary Patients

Antibiotic	Overall Success
Ampicillin	89%
Cephalexin	62%
Co-trimoxazole	81%
Trimethoprim	**96%**

ipral simple treatment for acute infections – 2 tablets b.d.

ipral for routine use in chronic U.T.I. – 1 tablet b.d.

In a study on recurrent U.T.I.'s
"... trimethoprim is the most effective of the antibacterial agents examined."[3]

Greater efficacy with fewer side effects.

In the trial in acute U.T.I.'s

"... the total side effects caused by ampicillin, cephalexin and co-trimoxazole were similar and occurred twice as often as when trimethoprim was given alone ..."[2]

Antibiotic	No. of Patients	% Side Effects
Ampicillin	88	22
Cephalexin	84	25
Co-trimoxazole	83	21
Trimethoprim	**84**	**8**
Total	339	

Prescribing information

IPRAL tablets
Each tablet contains trimethoprim B.P. 100 mg.

Indications
Ipral is indicated for the treatment and prophylaxis of acute and chronic urinary tract infections

Actions
Ipral is active in-vitro against most pathogenic Gram-positive and Gram-negative bacteria. Exceptions include Neisseria species and Pseudomonas aeruginosa.

Dosage and administration
For acute urinary tract infections 2 tablets twice daily is recommended
For long term and prophylactic therapy 1 tablet twice daily is recommended

Contra-indications
Ipral should not be given to patients with severe renal insufficiency where blood levels cannot be monitored regularly. Ipral should not be used in pregnancy or during the first few weeks of life

Precautions
Care is necessary in administration to patients with impaired renal function. Special precaution should be exercised in patients with a predisposition to folate deficiency

Side-effects
Nausea, vomiting and skin rashes may occur but are rare. Ipral may depress haematopoiesis during prolonged therapy. This has proved reversible when treated with calcium folinate.

Treatment of overdosage
Gastric lavage. Calcium folinate may be used to counteract any effect of Ipral on bone-marrow.

Package quantities and price
Packs of 100 tablets £5 30
Product Licence No - 0034/0190.

Full prescribing information available from:
Technical Services Department, E R Squibb & Sons Ltd.
Regal House, Twickenham, TW1 3QT
Tel: 01-892 0164

5280 Oct 79
Ipral is a Trade Mark of E R Squibb & Sons Ltd

...the way to effective treatment of urinary tract infections

- As effective as trimethoprim/sulphamethoxazole combination
- Avoids the side effects of sulphonamides
- At a sensible price

and "Trimethoprim given alone was shown to be much better tolerated."[2]
"Treatment with TMP (trimethoprim) alone is superior to the combined regimen due to a smaller incidence of side-effects; ..." thus "... introduction of TMP alone on the market is recommended."[4]
"Less gastrointestinal side-effects and skin rashes have occurred with trimethoprim ..."[3]

New **ipral**™
trimethoprim 100 mg
more than half the story

References
1. Lancet 1979, 1, 493 2. Br med. J. 1972, 2, 673.
3. Ann clin. Res. 1978, 10, (suppl. 22). 4. Chemother. 1973, 19, 314.

SQUIBB™ Full prescribing information available from Technical Services Department, E. R. Squibb & Sons Ltd Regal House, Twickenham, TW1 3QT Tel: 01-892 0164

MINOCIN

Minocycline
Hydrochloride
100 mg

TABLETS

for the treatment of gonorrhoea and non-specific urethritis[1-4]

1 Brit. J. Vener. Dis. (1975), 51, 97–98
2 Brit. J. Vener. Dis. (1975), 51, 206–209
3 Brit. J. Vener. Dis. (1976), 52, 43–45
4 Brit. J. Vener. Dis. (1978), 54, 107–111

INFORMATION ON THE USE OF MINOCIN IN THE TREATMENT OF VENEREAL DISEASE AND PRODUCT DATA WILL BE SUPPLIED UPON REQUEST

Lederle Laboratories
Research for British Medicine
A division of Cyanamid of Great Britain Ltd
Fareham Road, Gosport, Hants PO13 0AS

MINOCIN is a trademark for minocycline hydrochloride

British Journal of Venereal Diseases, 1980, 56, 1-2

The International Union against the Venereal Diseases and Treponematoses

R D CATTERALL*
From James Pringle House, Middlesex Hospital, London

Foundation

The International Union against the Venereal Diseases was founded in January 1923 as a result of the increased incidence of the venereal diseases following the First World War. The initiative was taken by the League of Red Cross Societies, the Belgian Association against Venereal Diseases, various social hygiene associations, but particularly by Professor Bayet of Belgium, who organised the initial negotiations in Brussels. The inauguration and first General Assembly was held in Paris, where the headquarters of the Union was established and delegates from 19 nations took part. The objectives of the Union were to co-ordinate the activities of voluntary organisations throughout the world concerned with social measures for the control of venereal diseases.

The early days

From its inauguration in 1923 until the onset of the Second World War in 1939, meetings were held regularly on alternate years in major cities such as Brussels, The Hague, London, Paris, Madrid, Budapest, Cologne, Algiers, and Cairo. During the early period of its existence the Union became widely known as a forum for discussion of methods of control. These included the adoption of suitable laws, the establishment of appropriate clinical services, education of the medical and paramedical professions, and public health education in the subject.

It is interesting to note that contact-tracing, which is now recognised as essential in the control of the diseases, was unknown 50 years ago, and the records of the Union indicate that this measure was vehemently criticised at the time.

Sex education also caused great problems and was difficult to establish because of enormous prejudice against the subject. However, the spread of venereal diseases among seamen was frequently discussed and the contribution of the Union to the application of the International Agreement of Brussels (1924) in respect of facilities for free treatment for all merchant seamen infected with a venereal disease was considerable.

At that time there was no general agreement about the problem of prostitution. Early in its existence the Union took a strong position against coercion and recommended the repeal of laws and regulations of that type. In their place it recommended measures based on general epidemiological principles applied to the whole population.

The postwar period

The activities of the Union started again in 1947 after the Second World War. The first post-war reunion was held in Paris in 1947, at which the governments and national societies of 32 countries took part. Since then the executive committee has met regularly every year in a major centre to plan the policy of the Union and to arrange the details of the forthcoming General Assembly. General assemblies have been held at two-yearly or three-yearly intervals in Copenhagen, Rome, Paris, Rotterdam, Naples, London, Stockholm, Washington, Lisbon, Munich, Budapest, Venice, Malta, and Leeds.

The Union was founded to co-ordinate activities on the social aspects of the control of venereal diseases and its objectives have been to stimulate the interest and assist the activities not only of those physicians practising the subject but also of epidemiologists, psychiatrists, psychologists, public health nurses, social workers, community physicians, and others concerned with human behaviour and its effect on the spread of venereal diseases. However, as a result of the great advances that have been made in medical knowledge and the appreciation that many diseases, other than the classical venereal diseases,

*President of the International Union against the Venereal Diseases and Treponematoses

Address for reprints: Dr R D Catterall, James Pringle House, Middlesex Hospital, London W1A 8AA

Received for publication 22 August 1979

are spread by the sexual route, the recent programmes of the General Assembly have been more scientifically and clinically orientated than in the past and the Union has become the major world forum for the dissemination of scientific, clinical, and therapeutic knowledge about the sexually transmitted diseases. At the General Assembly in Leeds in 1978 there were 210 delegates from 25 countries.

Co-operation and expansion

In 1948 the World Health Organisation invited the Union to become one of its official advisory bodies, and during the past 30 years there has been a close and harmonious relationship between the two organisations. The effectiveness of the collaboration between the Union and the World Health Organisation has been greatly strengthened by the new criteria established by WHO in respect of non-governmental organisations.

The expansion of health programmes in developing countries in Africa and Asia and the recognition of the importance of some non-venereal treponemal diseases, such as yaws and endemic syphilis, led to increasing consultations between WHO and the Union. Many yaws eradication programmes were planned and it was decided to modify the name of the Union to include this aspect of the work. At the General Assembly in Rotterdam in 1953 the new name of the Union was adopted and it became the International Union against the Venereal Diseases and Treponematoses.

Later it was increasingly apparent that the activities of the Union should be decentralised and that regional branches should be established, as far as possible along the lines of the regions already established by WHO. There are now five regional branch offices, one in Europe, North America, Latin America, South-east Asia and the Western Pacific, and, very recently, in Africa. Regional offices have the task of stimulating local action and adapting general policy to local needs. They should also encourage new members to join the Union, initiate the organisation of local meetings in the region, and foster the close collaboration of all those working in the subject within the region.

The Union has always tried to take a global view of the subjects on its agenda and the list of presidents indicates the international nature of its activities: Professor Bayet (Belgium), Professor Ehlers (Denmark), Professor Dubois (Switzerland), Dr Snow (United States), Professor Hermans (Holland), Dr Cavaillon (France), Dr Webster (United States), Dr King (Great Britain), Professor Canaperia (Italy), Dr Perdrup (Denmark), and Dr Catterall (Great Britain).

In the past, membership of the Union had consisted solely of organisations interested in the problems or actually working in the field. In 1962, however, it was decided to enlarge the categories of membership and to amend the statutes of the Union to introduce a new type of membership. This change enables all individuals who are interested in the work of the Union and wish to support its activities to become full members in their own right and an increasing number of individual members have joined in recent years.

The fiftieth anniversary of the founding of the Union was celebrated in Brussels in May 1973 at a joint meeting of the Union, the Belgian League against Venereal Diseases, and the Medical Society for the Study of Venereal Diseases.

The future

During the last half-century the activities of the Union have gradually expanded, despite some temporary fluctuations. The Union has become a truly international organisation and it has been able to influence events in many parts of the world and to provide help and expert guidance when needed. The General Assembly of the Union,* which is held every second year, has become the major international conference for those interested in the control of the venereal diseases and treponematoses. The continuing high prevalence of infections throughout the world, and the expansion of the subject to include the many other sexually transmitted diseases, has increased the need for exchange of information and for discussion between all those working in the field, including doctors, nurses, sociologists, and medical administrators.

Membership† of the Union provides a unique opportunity to meet, discuss, and collaborate with experts from all parts of the world and to keep up to date with modern trends and developments.

*A notice about the next General Assembly of the Union appears at the end of this issue (p 57)

†Inquiries about membership of the Union should be addressed to the Secretary General: Professor Anton Luger, Krankenhaus der Stadt Wein-Lainz, 13 Wolkenbergenstrasse, A-1130 Vienna, Austria (telephone no 82 26 11); or to the Secretary at the regional office.

British Journal of Venereal Diseases, 1980, **56**, 3-5

Characterisation of *Neisseria gonorrhoeae* from women with simultaneous infections at two sites

ROBERT C NOBLE

From the Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky, USA

SUMMARY Infection with two strains of *Neisseria gonorrhoeae* was found in paired cultures from 8·7% (6/69) of women who had infections of the cervix, rectum, or urethra. Paired gonococcal cultures from each patient were characterised by auxotyping, susceptibility to four antibiotics, and acrylamide gel electrophoresis patterns of the gonococcal cell proteins.

Introduction

Gonococci may be isolated simultaneously from more than one site in women with gonorrhoea when cultures are taken from the urethra, cervix, and rectum.[1] Rein *et al*[2] studied women with gonococcal infection of the rectum. In 40% of 3183 women treated for gonorrhoea, rectal infection accompanied cervical infection. Thirty-one per cent of women with infection of both cervix and rectum harboured different gonococcal strains on the basis of their different antibiotic susceptibilities. From Rein's[2] collection of gonococci six paired isolates with different antibiotic susceptibilities also had different gonococcal auxotypes as well as different acrylamide gel electrophoresis patterns of the gonococcal cell proteins.[3] These results suggested that possibly one-third of women with gonococcal infection of the cervix and rectum were infected with more than one strain of *Neisseria gonorrhoeae.*

The present study was designed to estimate the frequency with which different gonococcal strains were present at the same time in paired genitorectal cultures of women with gonorrhoea. Paired cultures were examined by means of gonococcal auxotyping, susceptibility to four antibiotics, and patterns produced by acrylamide gel electrophoresis of solubilised gonococcal cell proteins.

Patients and methods

BACTERIAL ISOLATES

The isolates were taken from 69 women (45 white, 23 black, 1 race unknown) with uncomplicated gonorrhoea attending the Lexington-Fayette County Health Department Venereal Disease Clinic. The patients were selected because at one clinic visit cultures giving positive results had been taken from two of the following sites: cervix, rectum, or urethra. The specimens were isolated on modified Thayer-Martin medium and were identified by bacterial morphology, Gram stain, oxidase reaction (1% solution of N, N-dimethyl-p-phenylenediamine monohydrochloride), and the production of acid with dextrose- but not maltose-, lactose-, sucrose-, or fructose-containing media. GC medium base (Difco Laboratories, Detroit, Michigan) with 1% IsoVitalex (Baltimore Biological Laboratory, Cockeysville, Maryland) was used as the culture medium for the study and for identification of the colonial morphological types. These were identified by observation of the bacterial colonies in indirect light.[4] Studies of the paired isolates were performed on a single colonial type. Gonococcal isolates were stored at −70°C in tryptic soy broth (Difco) containing 13% glycerol.

ANTIBIOTIC SUSCEPTIBILITY TESTS

Antibiotic susceptibility tests were performed using the plate dilution technique.[5] Reference micro-organisms with known antibiotic susceptibilities were included as controls in each test. The antibiotics (purchased from the United States Pharmacopeial Convention Inc., Bethesda, Maryland) were tested by means of two-fold dilutions in agar (ampicillin and penicillin 0·007-2 μg/ml, tetracycline 0·09-25 μg/ml, and spectinomycin 0·39-100 μg/ml). Antibiotic susceptibility results of paired isolates were judged to be alike if the test results were within two dilutions in the two isolates.

Address for reprints: Dr R C Noble, Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky 40536, USA

Received for publication 12 March 1979

GONOCOCCAL AUXOTYPING

Gonococcal auxotyping was performed by the method of Catlin[6] and Carifo and Catlin.[7] Auxotyping was performed twice on each isolate and gonococci of a known auxotype were included in each test.

ACRYLAMIDE GEL ELECTROPHORESIS

Acrylamide gel electrophoresis of the cell proteins of the gonococcal isolates was performed as previously described.[3] Overnight cultures of gonococci were lyophilised, heated in lauryl sulphate with 2-mercaptoethanol, and the solubilised cell proteins were subjected to alkaline sodium dodecyl sulphate gel electrophoresis. The resulting electrophoresis patterns were scanned with a spectrophotometer, and the comparisons of the gel scans were made by superimposing pairs of scans on a view box. The gel patterns of the two scans were judged to be the same if the configuration of the peaks coincided or if in one of the two patterns a single additional peak was present in the region corresponding to a protein with an estimated molecular weight of 13 000.[3]

ANALYSIS OF RESULTS

Two gonococcal isolates were considered to be of the same strain if they had the same auxotypes, antibiotic susceptibility test results, and protein electrophoresis patterns. If there were discrepancies in any one of these results, the isolates were said to be of different strains. Categorical data were analysed by the χ^2 method with Yates's correction.

Results

The number of cultures for gonococci giving a positive result were taken from paired sites as follows: cervix-rectum 41, cervix-urethra 25, urethra-rectum 1, and cervix-rectum-urethra 2. The auxotypes of the individual isolates were as follows: Zero 58, Pro 41, Arg 10, Arg-Hyx-Ura 21, Arg-Orn 2, Pro-Arg-Hyx-Ura 2, Arg-Orn-Hyx-Ura 2, and Pro-Arg 2. Gel electrophoresis was performed on all but nine of the 69 pairs of gonococcal isolates. One gonococcal strain was present in paired isolates from 63 of the 69 patients. Two gonococcal strains were found in the paired isolates of six of the 69 patients. The characterisation of the strains from these six patients are shown in the Table. Different gonococcal strains simultaneously infecting genitorectal sites were found in 8·7% (2-15·4%, 95% confidence interval) of the patients. Infection with more than one gonococcal strain was present in 9·7% (4/41) of the cervical-rectal pairs and 8% (2/25) of the cervical-urethral pairs. This difference was not significant when tested by the χ^2 method. Comparisons of the protein electrophoresis patterns were at variance with the auxotyping and the antibiotic susceptibility results in only one of the pairs in this study (patient 1, see Table). The two isolates from this patient had the same protein electrophoresis pattern despite differences in the auxotypes and the antibiotic susceptibilities.

Discussion

Other investigators have detected infections with more than one gonococcal strain in a single patient by means of differences in gonococcal antigens, gonococcal auxotypes, and the combination of auxotyping and antibiotic susceptibility testing.[8-10] The present study may be compared only with that of Catlin and Pace,[8] who used similar methods of

TABLE *Results of auxotyping, gel electrophoresis, and antibiotic susceptibility tests of pairs of gonococci isolated simultaneously from rectal and genital sites in six patients*

					Minimum inhibitory concentration (μg/ml)			
Patient No	*Site*	*Code No*	*Auxotype*	*Gel scan*	*Ampicillin*	*Penicillin*	*Spectinomycin*	*Tetracycline*
1	Cervix	5223	Zero	+	0·125	0·125	12·5	0·19
1	Urethra	5224	Pro		0·03	0·007	1·56	0·09
2	Cervix	5945	Pro	ND	0·03	1·0	6·25	0·19
2	Urethra	5938	Pro		0·50	0·50	12·5	0·19
3	Cervix	6559	Arg-Hyx-Ura	ND	NG	NG	6·25	0·19
3	Rectum	6560	Zero		0·06	0·25	6·25	1·56
4	Cervix	6506	Zero	–	0·125	0·125	3·12	0·39
4	Rectum	6508	Zero		0·03	0·03	NG	NG
5	Cervix	6418	Pro	–	0·03	0·03	6·25	0·39
5	Rectum	6419	Pro		NG	0·125	1·56	0·19
6	Cervix	6337	Pro	–	0·125	0·25	3·12	0·19
6	Rectum	6336	Pro		0·03	0·03	6·25	0·19

+ Agreement between pairs
– Disagreement between pairs
NG No growth
ND Not done

characterising gonococci. They identified two gonococcal strains in paired isolates of six (8·8%) of 68 women with infections of the cervix and rectum. However, three of the six pairs reported as different had identical auxotypes and the antibiotic susceptibility test results of these pairs differed by less than two dilutions. Thus, on the criteria of the present study, only 4·4% (3/68) of the paired isolates would have been different.

Selection of multiple colonies from the same culture plate might identify more patients with infections with two or more gonococcal strains. Johnston *et al*[9] identified gonococci with two or more serotypes in five patients by serotyping 15-20 bacterial colonies from a single culture plate. However, this study did not attempt to estimate the frequency of infection with multiple serotypes. Short *et al*[10] examined the auxotypes of eight gonococcal colonies picked from single cultures of each of 40 patients. More than one gonococcal auxotype was identified in 10 patients. However, this finding may not be comparable with that of the present study since they used a different typing method from that of Catlin.[6]

Simultaneous infections with multiple types of a single species of a Gram-negative organism are not limited to gonococcal infections. Three or four strains of *Escherichia coli* may be present in the stool specimens of healthy individuals.[11] Different *E coli* serotypes were also found in 20% of paired urine and faecal cultures from 98 patients with urinary tract infections.[12] Similarly, 17% of 75 patients with *Salmonella* infections of the intestine had multiple serotypes in their stool cultures.[13]

Although gonococcal infection of the urethra or rectum in women may possibly result from contact with infected vaginal secretions, infection with two or more gonococcal strains may be the result of vaginal or rectal intercourse with two or more infected sexual partners or both. Rectal intercourse was previously reported by 6·2% (10/161) of women attending the clinic of the present study.[14] Unfortunately, neither this study nor previous studies have detailed epidemiological data on the number or nature of sexual contacts. Thus, variations between the results of the studies mentioned above and those of the present study may reflect the frequency of sexual contact of women patients with different infected partners. Studies of gonorrhoea in women prostitutes or studies which examine a larger number of culture samples from each patient might indicate a higher percentage of patients infected with several gonococcal strains.

The author gratefully acknowledges the technical assistance of Mrs Betty R Miller, the suggestions of Dr Ernst Stolz and Dr A P Orange, Department of Dermatology, and the advice of Dr R van Strik, Department of Biostatistics, Erasmus University, Rotterdam. The co-operation of Dr Philip G Weiler, Commissioner of Health, Lexington-Fayette County Health Department, made study of the gonococcal strains possible. The reference strains for the antibiotic susceptibility tests (*N gonorrhoeae,* F18 and F29 and *Sarcinia lutea* F20) were generously provided by Dr Clyde Thornsberry of the Center for Disease Control, Atlanta, Georgia. The author also thanks Dr B Wesley Catlin of the Medical College of Wisconsin for the gonococcal strains of known auxotype (Thi, Pro-Hyx, Zero, Pro-Met-ThPP). Funds for this study were provided in part by a grant from the National Institutes of Allergy and Infectious Diseases, AI-11304, and by a grant from the Alexandrine and Alexander Sinsheimer Fund.

References

1. Schmale JD, Martin JE, Domescik G. Observations on the culture diagnosis of gonorrhea in women. *JAMA* 1969; **210:** 312-4.
2. Rein MF, Jaffe HW, Reynolds GH, Thornsberry C, Zaidi A, Wiesner PJ. Rectal gonococcal infection in women. *Abstract No. 80.* American College of Physicians 57th Annual Session, Philadelphia Pa, 1976.
3. Noble RC, Schell SC. Acrylamide gel electrophoresis of proteins of *Neisseria gonorrhoeae* as an epidemiologic tool. *Infect Immun* 1978; **19:** 178-86.
4. Kellogg DS, Peacock WL, Deacon WE, Brown L, Pirkle CI. *Neisseria gonorrhoeae* I Virulence genetically linked to clonal variation. *J Bacteriol* 1963; **85:** 1274-9.
5. Jaffe HW, Biddle JW, Thornsberry C, *et al.* National gonorrhea therapy monitoring study. In vitro antibiotic susceptibility and its correlation with treatment results. *N Engl J Med* 1976; **294:** 5-9.
6. Catlin BW. Nutritional profiles of *Neisseria gonorrhoeae, Neisseria meningitidis,* and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. *J Infect Dis* 1973; **128:** 178-94.
7. Carifo K, Catlin BW. *Neisseria gonorrhoeae* auxotyping. Differentiation of clinical isolates based on growth responses on chemically defined media. *Appl Microbiol* 1973; **26:** 223-30.
8. Catlin BW, Pace PJ. Auxotypes and penicillin susceptibilities of *Neisseria gonorrhoeae* isolated from patients with gonorrhoea involving two or more sites. *Antimicrob Agents Chemother* 1977; **12:** 147-56.
9. Johnston KH, Holmes KK, Gotschlich EC. The serological classification of *Neisseria gonorrhoeae.* I Isolation of the outer membrane complex responsible for serotypic specificity. *J Exp Med* 1976; **143:** 741-57.
10. Short HB, Ploscowe VB, Weiss JA, Young FE. Rapid method for auxotyping multiple strains of *Neisseria gonorrhoeae. J Clin Microbiol* 1977; **6:** 244-8.
11. Sears HJ, Brownlee I, Uchiyama JK. Persistence of individual strains of *Escherichia coli* in the intestinal tract of man. *J Bacteriol* 1950; **59:** 293-301.
12. Vosti KL, Goldberg LM, Monto AS, Rantz LA. Host-parasite interaction in patients with infections due to *Escherichia coli* I The serogrouping of *E coli* from intestinal and extraintestinal sources. *J Clin Invest* 1964; **43:** 2377-85.
13. Juenker AP. Infections with multiple types of *Salmonella. Am J Clin Pathol* 1957; **27:** 646-51.
14. Noble RC, Kirk NM, Slagel WA, Vance BJ, Somes GW. Recidivism among patients with gonococcal infection presenting to a venereal disease clinic. *Sex Transm Dis* 1977; **4:** 39-43.

British Journal of Venereal Diseases, 1980, 56, 6-11

Homosexuality and venereal disease in the United Kingdom
A second study

BRITISH CO-OPERATIVE CLINICAL GROUP*

SUMMARY The proportion of homosexually acquired cases of primary and secondary syphilis in patients attending venereal disease clinics in the United Kingdom has risen from 42·4% to 54% over a six-year period. Similarly, over the same period, the proportion of homosexually acquired cases of gonorrhoea has risen from 9·8% to 10·9%. The increase in incidence of homosexually acquired infections in both diseases occurred in all areas but particularly in London. Thus the very considerable epidemiological importance of male homosexuals as a high-risk group should receive more, not less, emphasis.

Introduction

In 1971 a study was carried out by the British Co-operative Clinical Group of the proportion of cases of primary and secondary syphilis and of gonorrhoea reported in male patients in the venereal disease clinics of the United Kingdom believed to have been homosexually acquired.[1] The present paper reports a similar study undertaken in 1977 and compares the results of the two surveys.

As in the previous study, the venereologists in charge of the clinics were asked to provide information on the number of anorectal and penile infections believed to have been homosexually or heterosexually acquired; but, in the recent study, infections which may have been acquired orally were also recorded. The figures are again presented separately for England, Wales, and Scotland, and on this occasion include data from Northern Ireland. As before, the figures for England are broken down according to the population size of the towns or cities in which the participating clinics are situated.

Patients and methods

The geographical distribution of the participating clinics is shown in Table I, together with the population of the towns and cities in which the clinics are situated.

The distribution of clinics was similar in the two studies, although the 1977 survey is less complete for England as three major clinics which participated in the earlier study failed to produce data for the 1977 one—in two instances an unjustified reliance had been placed on computers.

CLINIC ATTENDANCES

In 1977, 1364 cases of primary and secondary syphilis and 33 826 cases of gonorrhoea were recorded (Table II). According to the 1977 study the number of cases of gonorrhoea in England was notably lower than in 1971, owing mainly to the non-participation of three

TABLE I *Distribution of clinics in relation to size of towns and cities*

	1971		*1977*	
Area and population size	*Clinics*	*Towns or cities*	*Clinics*	*Towns or cities*
England				
50 000 or less	42	42	40	40
50 001-100 000	48	48	57	57
100 001-500 000	35	35	33	33
500 001 or more	7	5	6	4
London	20	1	17	1
Total	152	131	153	135
Scotland	13	11	15	15
Wales	11	11	7	7
N. Ireland	ND	ND	3	3
Total	176	153	178	160

ND = no data

---

*A list of participatory clinics is given in the appendix

Address for reprints: Dr R R Willcox, St Mary's Hospital, London W2

Received for publication 3 January 1979

TABLE II *Number and source of infection of cases of primary and secondary syphilis and gonorrhoea for 1971 and 1977*

Area	*No of cases* 1971	1977	*Contact (1977)* Heterosexual	Homosexual	*% homosexually acquired* 1971	1977
Primary and secondary syphilis						
England	830	1193*	500	692	45·9	58·1
Scotland	52	105	71	34	13·5	32·4
Wales	42	57	49	8	9·5	14·0
N. Ireland	ND	9*	7	2	ND	22·2
Total	924	1364	627	736	42·4	54·0
Gonorrhoea						
England	32 328	29 501	26 088	3413	10·5	11·6
Scotland	2822	3478	3262	216	4·1	6·2
Wales	1092	591	548	43	4·0	7·3
N. Ireland	ND	256	211	15	ND	5·9
Total	36 242	33 826	30 139	3687	9·8	10·9

ND = no data
*One patient who denied sexual contact was excluded from the analysis

large clinics, but this is insufficient to conceal a marked increase in the number of cases of primary and secondary syphilis in 1977.

Results

PRIMARY AND SECONDARY SYPHILIS

Of the 1363 cases of primary and secondary syphilis included in the 1977 study, 625 (45·9%) were penile infections heterosexually acquired, 355 (26%) were penile infections homosexually acquired, and 366 (26·9%) were homosexually acquired anorectal infections. Oral contact was the only source of infection in 15 homosexuals and two heterosexuals (total 1·2%). In all, 54% of infections were homosexually acquired and 46% heterosexually acquired. The proportion was highest (58·1%) for the clinics in England, less in Scotland (32·4%) and Northern Ireland (22·2%), and lowest in Wales (14%) (Table II).

All these figures are higher than those in the 1971 study. In England, for example, the percentage of acknowledged homosexually acquired infections increased by 12·2% in the six years, in Wales by 4·5%, and in Scotland by 18·9%.

Homosexually acquired infections in relation to population

In England the data are very striking. The percentage of homosexually acquired syphilitic infections has risen in all areas, especially at clinics in towns or cities with populations of 100 000 or less, although this is less marked in London, where in 1971 the percentage was already very high (Table III).

In London, 372 (70·9%) of 525 cases of primary and secondary syphilis included in this study were treated at five clinics (at the Middlesex, St George's, St Mary's, West London, and Westminster Hospitals) situated in the West End of the city. In these five clinics the proportion of homosexually acquired infections was 76·9% compared with 37·9% in the other participating London clinics (Table III). These figures are very similar to those of 1971.

TABLE III *Number of cases of homosexually acquired syphilis in England according to population of towns and cities (1977)*

Population of town or city	Total No of infections	Homosexually acquired	*% homosexually acquired* 1977	1971
50 000 or less	69	30	43·5	9·5
50 001-100 000	130	61	46·9	28·6
100 001-500 000	311	181	58·2	30·4
500 001 or more	157	76	48·4	16·8
London				
West End clinics (5)	372	286	76·9	73·3*
Other clinics (12)	153	58	37·9	37·2†
Total (17)	1192	692	58·1	45·9

*Five West End and †15 other London clinics

Indeed the 286 homosexually acquired infections recorded in the five West End clinics represented 83·1% of the total of 344 homosexually acquired infections in London. On the other hand, the 86 heterosexually acquired infections recorded in the same five clinics represented only 47·5% of the total number of 181 infections acquired heterosexually (Table III).

Individual clinic experience

The mean number of heterosexually and homosexually acquired cases of syphilis seen in 1977 at each clinic according to the size of towns or cities is shown in Table IV.

In Northern Ireland, Wales, Scotland, and towns and cities of less than 100 000 population in England, the mean number of syphilitic infections acquired heterosexually each year exceeded those acquired homosexually, whereas in towns or cities of 100 001-500 000 population and in London the reverse was true (Table IV).

The mean number of cases of homosexually acquired primary and secondary syphilis for each clinic in 1977 is compared with that in 1971 in Table IV. A sizable increase in 1977 was apparent in all areas.

The mean number of heterosexually acquired infections per clinic in 1977 is also compared with those in 1971. These figures too have risen in all sectors but—apart from Wales—not as markedly as the homosexually acquired infections.

Active-to-passive ratio of homosexually acquired infections

In the 1971 study, of 392 cases of homosexually acquired primary or secondary syphilis 172 (43·9%) were in active homosexuals and 220 (56·1%) were in passive homosexuals—that is, anorectally acquired (an active-to-passive ratio of 0·78:1).

In the 1977 study, of 736 homosexually acquired infections 355 (48·2%) were penile infections, 366 (49·7%) were anorectal infections, and 15 (all in England) (2%) were acquired orally (active-to-passive plus oral ratio of 0·93:1), although in two other cases (0·3%) the oral route was involved together with one of the other sites.

Oral infections in heterosexuals

It is highly probable that many patients were not questioned about oral intercourse. Whereas 17 (2·3%) homosexually acquired infections involved the oral route alone or in combination with another site, only two (0·32%) of the 627 heterosexually acquired infections involved the oral route alone and two, with other sites (a total of 0·64%).

GONORRHOEA

Of the 33 826 cases of post-pubertal gonorrhoea recorded in the 1977 study, 30 115 (89%) were penile infections acquired heterosexually, 1904 (5·6%) were penile infections homosexually acquired, and 1726 (5·1%) were homosexually acquired anorectal infections. The oral route was the only source of infection in 57 homosexuals and 24 heterosexuals (total 0·2%). In all, 3687 infections (10·9%) were homosexually acquired and 30 139 (89·1%) were heterosexually acquired; the figure for heterosexuals contrasts markedly with the 46% for primary and secondary syphilis (Table II).

As in the 1971 study, the percentage of cases of gonorrhoea in known homosexuals was much higher in England than in Scotland and Wales (as is also now shown for Northern Ireland) and all available data show an increase in the percentage of homosexually acquired infections compared with the previous study in 1971 (Table II).

Homosexually acquired infections in relation to population

The relatively high incidence of homosexually acquired gonorrhoea in England was mainly accounted for by the London figures; elsewhere in England the proportion resembled that found in Scotland, Wales, and Northern Ireland (Table V).

As with syphilis, the incidence of gonococcal infections in male homosexuals in London was

TABLE IV *Mean number of cases of homosexually and heterosexually acquired primary and secondary syphilis per clinic (1971 and 1977)*

		Mean No of infections					
		Homosexually acquired			*Heterosexually acquired*		
Area	*No. of clinics (1977)*	*1971*	*1977*	*% increase of mean*	*1971*	*1977*	*% increase of mean*
England							
50 000 or less	40	0·05	0·75	1400·0	0·45	0·98	117·8
50 001-100 000	57	0·42	1·07	154·8	1·04	1·21	5·8
100 001-500 000	33	1·49	5·48	267·8	3·40	3·94	15·9
500 001 or more	6	2·43	12·67	421·4	12·00	13·50	12·5
London	17	14·50	20·28	39·9	8·85	10·75	20·3
Total	153	2·51	4·52	80·0	2·95	3·26	10·5
Scotland	15	0·54	2·27	320·4	3·46	4·73	36·7
Wales	7	0·36	1·14	68·4	3·45	7·00	102·3
N Ireland	3	ND	0·67		ND	2·33	
Grand total	178	2·23	4·13	85·2	3·02	3·52	16·6

ND = no data

concentrated in the five West End clinics already mentioned, where they comprised 28·7% of infections compared with 8·8% in 12 other participating clinics in the city (Table V).

TABLE V. *Number of cases of homosexually acquired gonorrhoea in England according to population of towns and cities (1977)*

Population of town or city	Total No of infections	Homosexually acquired	% homosexually acquired 1977	1971
50 000 or less	1481	76	5·1	5·2
50 001-100 000	4378	197	4·5	5·0
100 001-500 000	9178	681	7·4	5·6
500 001 or more	4168	193	4·6	3·1
London				
West End clinics (5)	6840	1963	28·7	27·6*
Other clinics (12)	3456	303	8·8	7·7†
Total	29 501	3413	11·6	10·5

*Five West End and †15 other London clinics

Although 66·4% of the total number of cases of gonorrhoea seen at the participating London clinics were treated at the five West End clinics, no less than 86·6% of the homosexuals with gonorrhoea in London were treated in the same clinics compared with 60·7% of the heterosexuals with gonorrhoea (4877 out of 8030).

Although the 1971 data were not exactly comparable, the trend was towards an increase in the proportion of known homosexuals with gonorrhoea in all London clinics.

Individual clinic experience

In 1977 the mean number of known heterosexually and homosexually acquired gonococcal infections per clinic in the different areas and according to the size of the town or city in which the clinics are situated in England is shown in Table VI. In England in both groups the mean number increases according to the population size.

The mean number of cases of homosexually acquired gonorrhoea per clinic in 1977 is compared with that in 1971 in Table VI. The increase in the incidence of gonorrhoea is much less than that of primary and secondary syphilis, especially in England.

The mean number of heterosexually acquired gonococcal infections per clinic is shown in Table VI. In England and Wales, unlike the number of homosexually acquired infections, there was an overall decrease in the mean number of infections per clinic although the 1977 figures for England are affected by the lack of data from three major clinics, one in the group of 500 000 or more population and two in London.

Active-to-passive ratio of homosexually acquired infections

In the 1971 study, of the 3549 cases of homosexually acquired gonorrhoea 1945 (54·8%) were penile infections and 1604 (45·2%) were anorectal infections (active-to-passive ratio of 1·2:1). At that time no data were collected on oral infections.

In the 1977 study, which included 3687 cases of homosexually acquired gonorrhoea, 1904 (51·6%) were penile infections, 1726 (46·8%) anorectal infections, and 57 (1·5%) solely orally acquired infections (active-to-passive plus oral ratio of

TABLE VI *Mean number of cases of homosexually and heterosexually acquired gonorrhoea per clinic (1971 and 1977)*

Area	No of clinics (1977)	Mean No of infections: Homosexually acquired 1971	1977	% increase of mean	Heterosexually acquired 1971	1977	% increase of mean
England							
50 000 or less	40	1·4	1·9	35·7	24·9	35·1	40·1
50 001-100 000	57	3·5	3·5	0	66·9	73·4	9·7
100 001-500 000	33	14·1	20·6	46·1	238·8	257·5	7·8
500 001 or more	6	28·9	32·2	11·4	912·6	662·5	−27·4
London	17	123·4	133·3	8·0	496·9	472·4	− 4·9
Total	153	22·3	22·3	0	190·4	170·5	−10·5
Scotland	15	8·2	14·4	75·6	208·2	217·5	4·5
Wales	7	4·0	6·1	52·5	95·3	78·3	−17·8
N Ireland	3	ND	5·0	ND	ND	80·3	ND
Grand total	178	20·2	20·7	2·5	185·8	169·3	− 8·9

1·07:1). In addition, there were 38 other cases of anorectal or penile infections (1%) in which oral contact had also occurred. The validity of this data is limited as it is by no means routine practice to take oral specimens for culture from either suspected contacts or those with known gonorrhoea, and thus the proportion of cases in which oral investigations were carried out is not known.

Oral infections in heterosexuals

Of the 30 139 heterosexually acquired cases of gonorrhoea, 30 115 (99·99%) were penile infections and only 24 were oral, although there were 75 other cases in which oral as well as urethral contact took place. The total number of oral infections was thus 99 (0·3%), but no information is available on the number of patients with urethral infections or of contacts of gonorrhoea without urethral infections who also had oral specimens taken for culture.

GONORRHOEA-TO-SYPHILIS RATIO

The gonorrhoea-to-syphilis ratios for heterosexually acquired infections are shown in Table VII; the overall ratio was 48·1:1. The gonorrhoea-to-syphilis ratio of only 5:1 for homosexually acquired infections was nearly one-tenth that of heterosexually acquired infections (Table VII).

TABLE VII *Ratio of cases of gonorrhoea to syphilis*

	*Ratio of No of infections of gonorrhoea to syphilis**	
Area	*Heterosexually acquired*	*Homosexually acquired*
England	52·2:1	4·9:1
Scotland	45·9:1	6·4:1
Wales	11·2:1	5·4:1
N. Ireland	34·4:1	7·5:1
Total	48·1:1	5·0:1

*Actual numbers of cases for each infection are given in Table II

Discussion

The reasons for the difference in the ratio of gonorrhoea to syphilis could be that homosexually acquired gonococcal infections are more successfully concealed, although the active-to-passive ratio of declared cases of 1·07:1 compared with 0·93:1 for primary and secondary syphilis does not support this hypothesis. Nevertheless, as in women, asymptomatic infections in passive homosexual men would be expected to be more common than in active homosexual and heterosexual men. Possibly, syphilis is regarded more seriously and contact-tracing carried out more thoroughly for cases of gonorrhoea in homosexuals than in heterosexuals, but it is difficult to see why this should be so.

Thus, some factor—either physical in homosexual acts or behavioural in terms of the frequency of exposure—may well be responsible. But gonorrhoea is more easily acquired from a single exposure than is syphilis, since the latter more often requires frequent contact with the same person. Does this still apply in a homosexual pattern? Or is gonorrhoea more difficult to acquire from an infected, passive homosexual man than from a heterosexual woman?

Possibly it is not the relative lack of gonorrhoea which is the crux of the matter but the great excess of syphilis. Why is syphilis acquired nearly 10 times more frequently by homosexuals than by heterosexuals? Certainly this difference poses an intriguing epidemiological problem.

Both studies leave unanswered the question of whether there has been a real overall increase in those practising homosexuality or merely an increase in infections admitted to have been homosexually acquired. The ratio of heterosexually acquired gonococcal infections to those of primary and secondary syphilis in the clinics in the recent study was 48·1:1 whereas that for homosexually acquired infections was only 5·1:1; this highly significant difference likewise needs to be satisfactorily explained.

Appendix

LIST OF PARTICIPATING CLINICS

ENGLAND

Under 50 000 Population

Ashton-under-Lyme, Aylesbury, Barnstaple, Bishops Stortford, Boston, Bridgwater, Bury St Edmunds, Canterbury, Dartford, Dorchester, Douglas (Isle of Man), Falmouth, Folkestone, Grantham, Grays, Great Yarmouth, Hereford, Kettering, King's Lynn, Leamington, Loughborough, Milton Keynes, Minster, Newport (Isle of Wight), Newquay, Penzance, Redhill, St Austell, Salisbury, Scarborough, Skegness, Taunton, Truro, Tunbridge Wells, Weymouth, Whitehaven, Winchester, Windsor, Workington, Yeovil. (Forty clinics in 40 towns or cities; total population 1 240 935.)

50 001-100 000 Population

Barnsley, Barrow-in-Furness, Bath, Burnley, Burton-on-Trent, Bury, Carlisle, Chelmsford, Cheltenham, Chester, Chesterfield, Colchester, Crewe, Darlington, Dewsbury, Doncaster, Eastbourne, Exeter, Gloucester, Gravesend, Grimsby, Guernsey (Channel Islands), Guildford, Halifax, Harrogate, Hastings, High Wycombe, Jersey (Channel Islands), Keighley, Lancaster, Lincoln, Lowestoft, Maidstone, Mansfield, Margate, North Shields, Nuneaton, Peterborough, Preston, Rochester, Rochdale, Rotherham, Rugby, St Albans, Scunthorpe, Stevenage, Southport, South Shields, Torbay, Wakefield, Warrington, Watford, Weston-super-Mare, Wigan, Woking, Worcester, Worthing. (Fifty-seven clinics in 57 towns or cities; total population 4 000 882.)

100 001-500 000 Population

Birkenhead, Blackburn, Blackpool, Bolton, Bradford, Brighton, Bristol, Cambridge, Coventry, Derby, Dudley, Huddersfield, Ipswich, Leicester, Luton, Middlesbrough, Newcastle, Northampton, Norwich, Nottingham, Oldham, Oxford, Plymouth, Portsmouth, Reading, Stockport, Stoke-on-Trent, Southampton, Southend, Sunderland, Walsall, Wolverhampton, York. (Thirty-three clinics in 33 towns and cities; total population 6 576 520.)

Over 500 000 Population

Leeds, Liverpool (two clinics), Manchester (two clinics), Sheffield. (Six clinics in four cities; total population 2 117 200.)

LONDON

Central Middlesex Hospital, Croydon Hospital, Dreadnought Hospital (Greenwich), Guy's Hospital, Holloway Prison, Eastern Hospital, Middlesex Hospital, Old Church Hospital (Romford), Royal Free Hospital, Royal Northern Hospital, St Bartholomew's Hospital, St George's Hospital, St Giles Hospital (King's College Hospital), St Helier Hospital (Carshalton), St Mary's Hospital, West London Hospital (Charing Cross), Westminster Hospital. (Seventeen clinics; population 7 281 080.)

SCOTLAND

Aberdeen, Ayr, Dumfries, Dunfermline, Dundee, Edinburgh, Falkirk, Fort William, Greenock, Glasgow, Hamilton, Inverness, Kirkaldy, Perth, Stirling. (Fifteen clinics in 15 towns or cities; total population 2 072 905.)

WALES

Aberystwyth, Cardiff, Carmarthen, Haverfordwest, Pontypridd, Port Talbot, Swansea. (Seven clinics in seven towns or cities; total population 567 100.)

NORTHERN IRELAND

Belfast, Coleraine, Londonderry. (Three clinics; total population 456 850.)

References

1. British Co-operative Group. Homosexuality and venereal diseases in the United Kingdom. *Br J Vener Dis* 1973; **49**; 329-34.

British Journal of Venereal Diseases, 1980, 56, 12-16

Recent observations on the serology of syphilis*

A LUGER, B SCHMIDT, I SPENDLINGWIMMER, AND F HORN

From the Department of Dermatology, City Hospital, Vienna-Lainz, and the Ludwig Boltzmann Institute, Vienna

SUMMARY Routine screening of 404 742 sera by the automated micro-haemagglutination assay (AMHA-TP) and the Venereal Disease Research Laboratory (VDRL) test showed that 9848 specimens gave a reactive result to one of the three assays. Reactive results were confirmed by the fluorescent treponemal antibody absorption (FTA-ABS) test. The possibility of false-positive results varied from 0·04-0·38% of all specimens or from 1·7-15·7% of reactive sera. The VDRL test failed to detect reactivity in 56·54% of sera from patients who had previously been infected with *Treponema pallidum*.

The importance of routine testing by the AMHA-TP is illustrated by the detection of four patients with mesaortitis and two with active neurosyphilis among a selected group of 54 patients who had non-reactive results to the VDRL test. Testing of cerebrospinal fluid specimens by the AMHA-TP test produced more specific results than by the other two tests.

Introduction

Recent research into the serology of syphilis has concentrated on the identification of specific IgM and IgG antibodies against *Treponema pallidum*. The basic fluorescent treponemal antibody absorption (FTA-ABS) test however allows a margin of error[1-5] which may be 6·8-10·7%,[6] 7%,[7] or 37·2%.[8] The sources of error may be non-specific group antibodies, the use of an inefficient sorbent, changes on the cell surface due to lyophilising of *T pallidum*, and to other factors.[4 9-12]

The occurrence of *T pallidum*-specific antibodies of the IgM class seems to indicate early or active syphilitic infection whereas their disappearance after antibiotic treatment may prove a cure.[10 11 13-16]

The methods used in these investigations are so far not precise enough for definite conclusions.[11 17 18] An improvement in methods is taking place in several research centres and promises a better approach in a few years.

The results of the *T pallidum* haemagglutination assay (TPHA) have been assessed by many workers in the last few years.[19-37] This test appears to be the most sensitive, specific, and economical method for the serodiagnosis of syphilis. It indicates the presence of IgM and IgG antibodies against *T pallidum* and may therefore become reactive before any other test.[23-25 37]

The micro-haemagglutination assay (MH-TP) and especially the automated version of this procedure (AMHA-TP) is most valuable for screening purposes.[12 19 23-25 27 38-43] The common trend in screening is towards the application of the AMHA-TP and a lipoidal antigen test, the Venereal Disease Research Laboratory (VDRL) test, the rapid plasma reagin (RPR) test,[24 27 40] the reagin serum (RST) test,[43] and others. At present however the VDRL test is the best standardised test in use based on 25 years' experience.

Methods

The use of the AMHA-TP test and the VDRL test for screening was first suggested in 1972 by Luger and Spendlingwimmer.[25] Since that time, 404 742 sera have been examined during a period of six years at the Department of Dermatology in the City Hospital Vienna-Lainz and evaluated by the Ludwig-Boltzmann Institute of Dermatovenereological Serodiagnosis. All samples were received from hospitals, outpatient dispensaries, or the central health office in Vienna. The sera were tested routinely by the AMHA-TP and by the VDRL test. Reactive results were checked by the FTA-ABS test. The tests were performed as described.[10 24 27]

---

*Paper presented at the Spring Meeting of the MSSVD at Leeds, 1978

Address for reprints: Professor A Luger, Krankenhaus der Stadt Wien Lainz, A-1130 Vienna, Austria

Received for publication 18 May 1978

Results and comments

The results of sera examined between 13 March 1972 and 30 April 1978, as well as of the reactivity to the three tests, are given in Table I. A total of 394 894 samples were non-reactive to the AMHA-TP and to the VDRL test and were therefore not investigated further. Of the remainder, 9848 sera were reactive to one or both of the two screening tests and the results were checked by the FTA-ABS test.

TABLE I *Results of serological tests on 404 742 sera*

Reactivity to following tests					
AMHA-TP	*FTA-ABS*	*VDRL*	*No of sera*	*% of total No reactive to 1/3 tests*	*% of total No examined*
+	−	−	1377	13·98	0·34
+	−	−	4226	42·91	1·04
+	+	+	3248	32·98	0·80
+	−	+	95	0·97	0·02
−	+	+	73	0·74	0·02
−	−	+	829	8·42	0·21
Total No reactive to 1/3 tests			9848	100·00	2·43
Total No non-reactive to AMHA-TP/VDRL			394 849		97·57
Grand total			404 742		100·00

+ Reactive − non-reactive

Of the 1377 sera showing reactive results to the AMHA-TP but non-reactive results to the FTA-ABS test and the VDRL test, a few may have occurred in patients at the beginning of the infection (before the third week) but, as far as could be traced, they were mainly found in patients who had contracted syphilis many years previously, were adequately treated, and were probably cured. Some sera came from old people who denied having had syphilis.

No history was obtainable from most of the donors. Further investigations to try to elucidate the reasons for an isolated reactive result to the AMHA-TP were undertaken but these results could have been non-specific (false-positive results) in several cases.

The results of the 4226 specimens which were reactive to the AMHA-TP and to the FTA-ABS test but not to the VDRL test indicate that the donors had syphilis, but the non-reactivity of the VDRL test suggests that the infection had occurred many years previously, had been treated successfully, or had disappeared spontaneously.

Since 3248 sera were reactive to the AMHA-TP, to the FTA-ABS, and to the VDRL tests, there can be no doubt the donors all had syphilis. Ninety-five specimens were reactive to the AMHA-TP and to the VDRL test but non-reactive to the FTA-ABS test, and 73 specimens were non-reactive to the AMHA-TP test but reactive to the FTA-ABS and VDRL tests. These latter two groups comprise results which showed either false-reactivity or false non-reactivity in one of the two tests using treponemal antigens. The results for the 829 sera which were non-reactive to the AMHA-TP and the FTA-ABS test but reactive to the VDRL test can be considered biological false-positive reactions.

The possibility of obtaining false results by the above method of screening may vary from 1·7 to 15·7% of the reactive samples and from 0·04 to 0·38% of all sera examined. The first figure constitutes an absolute minimum, the last an improbable maximum. The rate of specific reactivity of 1·84% (minimum) seems to be high but this is due to the selection of patients for serum antibody tests in most of the hospitals and dispensaries.

NON-REACTIVE RESULTS IN SELECTED GROUP

Results were obtained from 53 patients who were reactive to the AMHA-TP as well as to the FTA-ABS tests but non-reactive to the VDRL test. These patients were asked to attend for clinical examination. Of these, 10 had a history of a previous infection and had been adequately treated. Of the 43 who did not know that they had once had syphilis, 37 had no symptoms, four had mesaortitis with aortic diameters ranging from 40 to 50 mm on their radiographs (only one had attended the hospital because of cardiac complaints, one attended because of a leg ulcer, one had hepatic cirrhosis, and one had a keratoacanthoma), and two had changes of active neurosyphilis in the cerebrospinal fluid (one had tabes dorsalis and the other, clinically asymptomatic meningovascular syphilis with focal signs on electroencephalographs). Both these latter patients attended the department of urology, the first because he had difficulties of micturition (early tabetic paralysis of the bladder) and the other because of vague urological complaints. Late syphilis was discovered in all six patients by routine serum antibody tests.

These investigations could be performed on only 53 (1·25%) of 4226 patients and concern exclusively patients of the City Hospital, Lainz. These observations were therefore of a highly selected group and are not at all representative, although the results emphasise the importance of routine serological testing with the AMHA-TP instead of the VDRL test.

COMPARATIVE EVALUATION OF TESTS

In a comparative evaluation of the three tests (Table II), the agreement between the AMHA-TP and FTA-ABS test was 82·87% of the reactive samples or 99·61% of all sera (reactive plus non-reactive results); agreement between the AMHA-TP and the VDRL test was 33·95% or 98·39% respectively and

TABLE II *Comparative evaluation of results to three tests*

Reactivity to following tests	*Reactivity to following tests* FTA-ABS − No	− %	+ No	+ %	Total No	Total %	VDRL − No	− %	+ No	+ %	Total No	Total %
VDRL												
−			4226	49·89	4226	49·89						
+	924	10·91	3321	39·20	4245	50·11						
Total	924	10·91	7547	89·09	8471	100·00						
AMHA-TP												
−			73	0·81	73	0·81			902	9·15	902	9·15
+	1472	16·32	7474	82·87	8946	99·19	5603	56·90	3343	33·95	8946	90·85
Total	1472	16·32	7547	83·68	9019	100·00	5603	56·90	4245	43·10	9848	100·00

+ Reactive − non-reactive

between the FTA-ABS test and the VDRL test 39·2% or 98·72% respectively. The somewhat better agreement between the FTA-ABS and VDRL tests compared with the AMHA-TP and VDRL test can be explained by the fact that the FTA-ABS test sometimes (in primary and in early secondary syphilis) returns earlier to non-reactivity after adequate treatment than does the AMHA-TP.

The importance of the use of the AMHA-TP for screening is shown by the fact that the VDRL test alone failed to indicate reactivity in 4226 (56·54%) of 7474 sera which were reactive to both the AMHA-TP and the FTA-ABS test and thus came from persons who were infected at one time with *T pallidum*. The significance of detecting specific antibodies in the sera of these patients has been emphasised by the serological results of the 53 selected patients already mentioned.

REACTIVITY OF CSF SAMPLES

Little is known about the comparative reactivity of serum and cerebrospinal fluid (CSF) to the haemagglutination assay. Specimens from 238 donors were examined. The AMHA-TP (micro-method) was performed using serum and the TPHA (macro-method) using CSF. There was no difference in the method of examination of serum and CSF by the other two tests (FTA-ABS and VDRL).

COMPARATIVE EVALUATION

A comparative evaluation of the reactivity to the three tests on serum and CSF is summarised in Table III. The sera of two patients were non-reactive but the CSF was reactive to the AMHA-TP test. Both patients had clinical features of tabes dorsalis, one with markedly increased CSF protein (0·83 g/l) and a normal cell count (1/ml), the other with slightly

TABLE III *Comparative evaluation of serological test results for serum and cerebrospinal fluid*

Reactivity of serum to	*Reactivity of CSF to* TPHA − No	− %	+ No	+ %	Total No	Total %	VDRL − No	− %	+ No	+ %	Total No	Total %	FTA-ABS − No	− %	+ No	+ %	Total No	Total %
AMHA-TP																		
−			2	0·84	2	0·84												
+	84	35·29	152	63·87	236	99·16												
Total	84	35·29	154	64·71	238	100·00												
VDRL																		
− (+/−)							138	57·98			138	57·98						
+							85	35·72	15	6·3	100	42·02						
Total							223	93·70	15	6·3	238	100·00						
FTA-ABS																		
−													23	9·66			23	9·66
+													144	60·51	71	29·83	215	90·34
Total													167	70·17	71	29·83	238	100·00

+ Reactive; +/− weakly reactive; − non-reactive increased protein and a cell count of 4/ml. The serum and CSF of the first patient were both reactive to the haemagglutination test at a dilution of 1/40, which is a reactive result for CSF but a non-reactive result for serum. Both the serum and the CSF were non-reactive to the FTA-ABS and VDRL tests. The serum of the second patient was non-reactive to the haemagglutination assay and to the VDRL test; the FTA-ABS test result was borderline on several different attempts with material from the same sample whereas the CSF was reactive to the haemagglutination tests at a dilution of 1/40 and non-reactive to the FTA-ABS and VDRL tests.

The antibody titre, as detected by the AMHA-TP, was equally high in CSF and serum, which indicates that these antibodies have not necessarily come from the serum since the protein values did not indicate a breakdown of the blood-CSF barrier; thus they may have been produced within the cerebrospinal system.

Of the donors who were sero-reactive to the haemagglutination assay, 65·3% were also CSF-reactive to the same test. The figures for the FTA-ABS and VDRL tests were 33% and 15% respectively. This seems to indicate that the haemagglutination method is twice as sensitive as the FTA-ABS test and four times as sensitive as the VDRL test for CSF.

In a comparative quantitative evaluation of the results of the haemagglutination assay higher titres occurred in CSF in 11 (4·62%) of 238 samples and in serum in 224 (94·12%) of 238 samples. The VDRL titres were all higher in serum than in CSF; this suggests that the haemagglutination assay may sometimes be more sensitive in CSF than in serum when quantitatively evaluated. Further research is however necessary to improve the technique and reduce the margin of error.

References

1. Baertschy D, Golubjatnikov R, Stedman M, Inhorn LS. Serologic study of specimens with borderline FTA-ABS test reactivity. *Health Lab Sci* 1977; **14**: 177-82.
2. Burns RE. Spontaneous reversion of FTA-ABS test reactions. *JAMA* 1975; **234**: 617.
3. John JF, Cuetter AC. Spinal syphilis: the problem of fluorescent treponemal antibody in the cerebrospinal fluid. *South Med J* 1977; **70**: 309-11.
4. Shin DH, Kass MA, Kolker AE, Becker B, Marr JJ, Bell CE. Positive FTA-ABS tests in subjects with corticosteroid-induced uveitis. *Am J Ophthalmol* 1976; **82**: 259.
5. Zajac W, Pucilo K, Zaremba A. A serological analysis of weakly positive and doubtful results of tests for syphilis in elderly persons. *Przegl Dermatol* 1975; **62**: 205-9.
6. Leitgeb G, Cervetti O, Giovannini E, Sartoris S, Goitre M. Studio della lue primaria. *G Ital Derm* 1975; **110**: 83-92.
7. Drew FL, Sarandria JL. False-positive FTA-ABS in pregnancy. *J Amer Vener Dis Assoc* 1975; **1**: 165.
8. Dans PE, Judson FN, Larsen SA, Lantz MA. The FTA-ABS test: a diagnostic help or hindrance. *South Med J* 1977; **70**: 312-5.
9. Kiraly K, Kramer M. Die entwicklung der serologischen syphilis-diagnose während der letzten 30 Jahre. *Borgygy Vener Szle* 1976; **52**: 10-21.
10. Luger A, Schmidt B, Spendlingwimmer I. Quantitative evaluation of the FTA-ABS-IgM and VDRL test in treated and untreated syphilis. *Br J Vener Dis* 1977; **53**: 287-91.
11. Wilkinson AE, Rodin P. IgM-FTA test in syphilis in adults; Its relation to clinical findings. *Br J Vener Dis* 1976; **52**: 219-23.
12. Luger A. Recent developments in the serological diagnosis of syphilis. *Proceedings of the XV International Congress of Dermatology*. Amsterdam; Excerpta Medica, 1978.
13. Ghinsberg R, Grunbaum BW, Blumstein G. Rapid detection of specific antitreponemal antibodies by counterimmunoelectrophoresis using an extract of *Treponema pallidum*. *Isr J Med Sci* 1977; **13**: 557-60.
14. Müller F. Modellversuche zur wirkung von IgM und IgG antikorpern auf die reaktionen des *Treponema pallidum* hämagglutinations (TPHA) testes. *Hautarzt* 1976; **27**: 26-9.
15. Müller F. Serodiagnostik aus der sicht des immunologen. *Hautarzt* 1977; **28**: 167-72.
16. Wilkinson AE. Some aspects of research of syphilis. Serological evidence of activity of the disease. In: Catterall RD, Nicol CS, eds. *Sexually Transmitted Diseases*. London: Academic Press, 1976; 214-8.
17. Kerl H, Bayer U, Turek TD. Beurteilung und klinische bedeutung des *Treponema pallidum* hämagglutinations (TPHA) testes in der syphilisdiagnostik. *Z Hautkr* 1976; **51**: 718-26.
18. Kaufman RE, Olansky DC, Wiesner PJ. The FTA-ABS (IgM) test for neonatal congenital syphilis. A critical review. *J Amer Vener Dis Assoc* 1975; **1**: 79.
19. Adler MW, Belsey EM, O'Connor BH, Catterall RD, Miller DL. Facilities and diagnostic criteria in sexually transmitted disease clinics in England and Wales. *Br J Vener Dis* 1978; **54**: 2-9.
20. Alessi E, Scioccati L. Test di emoagglutinazione treponemica (TPHA). Validità del micrometode esognito con le diluizioni dei seri 1/20-1/40. *Boll Ist Seiroter Milan* 1977; **56**: 102-7.
21. Kiraly K, Prerau H. Evaluation of the *Treponema pallidum* haemagglutination (TPHA) test for syphilis on "problem sera". *Acta Derm Venereol (Stockh)* 1974; **54**: 303-10.
22. Lesinski J, Krach J, Kadziewicz E. Specificity, sensitivity, and diagnostic value of the TPHA test. *Br J Vener Dis* 1974; **50**: 334-40.
23. Luger A. Geschlechtskrankheiten. *Hautzart* 1977; Suppl 2, 12-21.
24. Luger A. Immunologie der syphilis. In: Korting GW, ed. *Dermatologie in Praxis und Klinik*. Stuttgart: G Thiemi, (in press).
25. Luger A, Spendlingwimmer I. Der *Treponema pallidum* hämagglutinations (TPHA) test. *Wien Klin Wochenschr* 1972; **84**: 657-63.
26. Luger A, Spendlingwimmer I. An appraisal of the *Treponema pallidum* haemagglutination (TPHA) test. *Br J Vener Dis* 1973; **49**: 181-2.
27. Luger A, Spendlingwimmer I. Der automatisierte mikrohämagglutinations test mit *Treponema pallidum* antigen (AMHA-TP test). *Hautarzt* 1974; **25**: 238-44.
28. Luger A, Spendlingwimmer I, Zips A, Ogris E, Hawlicek F, Puhringer R. Das verhalten der reaktivitat in syphilis serologischen untersuchungsmethoden. *Z Hautkr* 1976; **51**: 718-26.
29. Manikowska-Lesinska W, Linda B, Szymska K, Zajac W. Studies on the method of the TPHA test. *Przegl Dermatol* 1975; **62**: 663-9.
30. Muller F, Luger A. Treponemen-spezifische immunodiagnostik der syphilis und deren normierung. *Z Hautkr* 1976; **51**: 643-51.
31. O'Neill P. A new look at the serology of treponemal disease. *Br J Vener Dis* 1976; **52**: 296-9.
32. Robertson DHH, McMillan A, Young H, Henrichsen C. Clinical value of the *Treponema pallidum* haemagglutination test. *Br J Vener Dis* 1975; **51**: 79-82.
33. Schröter R. Die serodiagnostik der syphilis. *Ther Umsch* 1975; **33**: 9-15.
34. Shore N. Haemagglutination tests and related advances in serodiagnosis of syphilis. *Arch Dermatol* 1974; **109**: 854-7.

35. Willcox RR. Perspectives in venereology. *Abstr Hyg* 1976; **51:** 237-92.
36. Yogeswari L, Sowmini CN. *Treponema pallidum* haemagglutination test (TPHA) for the specific serodiagnosis of syphilis. *Indian J Dermatol* 1974; **40:** 185-92.
37. Jephcott AE, Beveridge MAM, Sequeira PJL. Early TPHA response in primary syphilis. *Br. J Vener Dis* 1977; **53:** 147.
38. Kern A. Notwendigkeit und kriterien einer zeitgemäßen routine such serodiagnostik der syphilis. *Medicamentum (Berlin)* 1975; **16:** 34-41.
39. Kippax PW, Okoye RC. Screening for treponemal infection. *Br J Vener Dis* 1975; **51:** 144.
40. Luger A, Spendlingwimmer I, Horn F. Der *Treponema pallidum* hämagglutinations test als suchreaktion. *Ther Umsch* 1976; **33:** 16-9.
41. Young H, Henrichsen C, Robertson DHH. *Treponema pallidum* haemagglutination test as a screening procedure for the diagnosis of syphilis. *Br J Vener Dis* 1976; **50:** 341-6.
42. Rudolph AH. The microhaemagglutination assay of *Treponema pallidum* antibodies (MHA-TP). *J Amer Vener Dis Assoc* 1976; **3:** 3-8.
43. Black DA, Ray PE, Therrell BL. Qualitative evaluation of the reagin screen test. *J Clin Microbiol* 1976; **4:** 16.
44. Bowszyc J. The cerebrospinal fluid in syphilis in the aspect of immunological studies. *Przegl Dermatol* 1975; **62:** 365-71.
45. Duncan WP, Kuhn USG. Further studies of the fluorescent treponemal antibody cerebrospinal fluid (FTA-CSF) test with a monospecific anti-IgM-conjugate. *Br J Vener Dis* 1973; **49:** 487-90.
46. Vakovleva N. A quantitative immunofluorescence method with the cerebrospinal fluid from patients with different forms of syphilis. *Vestn Dermatol Venereol* 1975; **2:** 31-6.
47. Zajac W, Krach-Karczmarczyk J, Pucilo K. Diagnostic value of serological tests in the cerebrospinal fluid. *Przegl Dermatol* 1973; **63:** 319-23.
48. Catterall RD. Neurosyphilis. *Br J Hosp Med* 1977; **17:** 585-604.
49. *Lancet.* Editorial: Routine tests for syphilis on cerebrospinal fluid. *Lancet* 1977; **2:** 595.

British Journal of Venereal Diseases, 1980, **56**, 17-19

Non-specific positive test results to syphilis in dermatological diseases

M GIBOWSKI AND E NEUMANN

From the Dermatological Clinic, Institute of Nervous System and Sensory Organ Diseases, Medical Academy, Poznan, Poland

SUMMARY Sera from 3028 patients attending a dermatological clinic were examined by the fluorescent treponemal antibody (FTA) test, the fluorescent treponemal antibody-absorbed (FTA-ABS) test, the Venereal Disease Research Laboratory (VDRL) test, and Kolmer's test. Eleven cases of late syphilis were found. Sera from 63 (2·08%) of the remaining 3017 patients showed non-specific results; these were found more frequently in patients with pyodermas, neoplasms, acne, mycoses, crural ulceration, and psoriasis than in those with other diseases. No non-specific results were observed in patients with bullous skin diseases and only a few in patients with viral skin diseases and chronic lupus erythematosus. The test producing the most non-specific results was the FTA test, followed by the FTA-ABS test, the VDRL test, and Kolmer's test.

Introduction

The problem of false-positive results to treponemal antibody tests occurring in patients without syphilis has been considered by many investigators. Most authors have studied healthy subjects, such as blood donors, students, and military recruits,[1-10] patients with collagenoses—particularly lupus erythematosus[4 6 7 11-16]—patients with systemic diseases and with bacterial or viral infections[4 6-8 15] and other diseases. There are only a few reports on non-specific positive results occurring in patients with skin diseases. This problem has been investigated mainly in diseases which interest general physicians and dermatologists, such as lupus erythematosus, vascular changes, leprosy, and sensitivity to drugs.[6 11 13 17] We have studied patients with a wider range of skin diseases.

Patients and methods

The sera of patients attending the Dermatological Clinic of the Medical Academy in Poznan were examined between 1974 and 1977. A total of 3028 men and women was studied. All sera were examined by the qualitatative fluorescent treponemal antibody-absorbed (FTA-ABS) test, the quantitative fluorescent treponemal antibody (FTA) test, the Venereal Disease Research Laboratory (VDRL) test, and Kolmer's test. Those sera giving reactive results to the FTA-ABS test were also examined by the *Treponema pallidum* immobilisation (TPI) test. Unexpectedly reactive results were accepted only after repeat examination of the same serum sample and after examination of another sample from the same patient. The FTA-ABS and FTA tests were regarded as giving positive results when the intensity of fluorescence was + + to + + + +. Fluorescence of + and +/+ + was regarded as giving weakly positive results. All tests were carried out by the methods routinely applied in Poland and described in detail by Lesiński and his co-workers.[18]

Results and discussion

Of the 3028 patients studied, 11 (0·36%) had late syphilis. All these patients had negative results to cardiolipin tests and syphilis was suspected on the basis of reactive results to fluorescent tests confirmed by the TPI test. (Among those 11 cases there were 10 with late latent syphilis and one with late congenital syphilis.)

The remaining patients were divided into categories according to the types of diseases (Table). Of these, 63 (2·08%) had sera showing non-specific reactivity to at least one test. The highest proportion of these results was among patients with pyodermas

Address for reprints: Dr M Gibowski, Klinika Dermatologii, Narzadow Zmyslow AM w Poznaniu, 60-355 Poznan, ul Przybyszewskiego 4, Poland

Received for publication 26 March 1979

TABLE *Non-specific positive results to serological tests in dermatological diseases*

Disease category	*No of patients*	*Total*	*With >1 positive or weakly positive test result*		*Reactive results to following tests*								
					FTA-ABS			*FTA*			*VDRL*	*Kolmer's*	
			No	*%*	+ +	+	*Total (%)*	+ +	+	*Total (%)*	+ +	+ +	*Total (%)*
Allergic diseases		933	15	1·60	2	2	4 (0·43)		11	11 (1·17)	3	1	4 (0·43)
Dermatitis	745												
Urticaria	151												
Prurigo	38												
Collagenoses		207	2	0·97					2	2 (0·97)			
Chronic lupus erythematosus	160												
Scleroderma	47												
Bullous skin diseases		75											
Dermatitis herpetiformis	52												
Pemphigus vulgaris	8												
Pemphigoid	5												
Skin neoplasms		369	13	3·52	1	4	5 (1·35)		10	10 (2·71)	1	1	2 (0·54)
Epithelial	286												
Ca spinocell	69												
Keratoacanthoma	8												
Fungoid	6												
Psoriasis		602	12	2·0	2	2	4 (0·66)		9	9 (1·50)		2	2 (0·33)
Acne vulgaris	74												
and rosaceae	76	150	5	3·3	1		1 (0·66)	1	4	5 (3·33)			
Ulcera crurum		193	4	2·8				1	2	3 (1·55)	1		1 (0·52)
Purulent skin diseases		73	4	5·50		2	2 (2·74)		3	3 (4·11)	1		1 (1·37)
Erysipelas	49												
Pyodermia	24												
Viral skin diseases		77	1	1·30				1		1 (1·30)			
Herpes zoster	42												
Herpes vulgaris	15												
Condylomata accuminata	20												
Dermatomycosis		119	3	2·52	2		2 (1·68)		3	3 (2·52)	1		1 (0·84)
Other skin diseases		219	4	1·82	1	1	2 (0·92)	1	2	3 (1·37)	1	1	2 (0·92)
Total		3017	63	2·08	9	11	20 (0·66)	4	46	50 (1·65)	8	5	13 (0·43)
Percentage				2·08	0·30	0·36	0·66	0·13	1·52	1·65	0·26	0·17	0·43

+ + to + + + + = positive
+ and +/+ + = weakly positive

(5·5%) followed by skin neoplasms (3·52%), acne vulgaris and rosacea (3·33%), mycoses (2·52%), crural ulceration (2·08%), and psoriasis (2%). Patients with bullous diseases were the only category in which no non-specific results were found; this group consisted mostly however of patients with dermatitis herpetiformis (52 patients); the other bullous diseases were less common. Only a few non-specific results were found in patients with viral skin diseases and collagenoses. It is interesting that we found comparatively few non-specific results in the group of patients with collagenoses, since many authors have reported non-specific results occurring solely in these diseases.[6 13 16]

In our study no positive results to cardiolipin tests were found in the 160 patients with lupus erythematosus but two sera with weakly positive results (+) were found by the FTA test. All our patients however had chronic lupus erythematosus and most of them had been treated for a long time, whereas most of the patients of the authors mentioned above were systemic cases (SLE) and untreated cases. Similarly in the group of patients with viral diseases only a few non-specific results were obtained. Wright *et al*[19] observed 13 non-specific results to the FTA-ABS test in 32 patients with herpes genitalis and Chapel *et al*[20] found only one case with a non-specific result to the FTA-ABS test and one with a positive result to the VDRL test in 44 patients with the same disease. We did not have any such cases; most of our patients had herpes zoster. Fifteen patients had herpes simplex and in all of them the fluorescent and cardiolipin tests gave negative results.

Among the 63 patients with non-specific results were nine, who had—in addition to their skin condition—systemic diseases such as pneumonia, fibrous and cirrhotic changes in the lungs, diabetes, tuberculosis, glomerulonephritis, and chronic gonococcal tonsillitis. (Of the nine patients, two had positive results to the FTA-ABS test, three weakly positive results to the FTA test, and two positive results to Kolmer's test.)

For comparison, Johansson[12] found that of 6737 patients attending a dermatological department 0·4% had false-positive results to four cardiolipin tests. In our investigations using two cardiolipin tests the percentage was similar (0·43%). Sarandria[9] found that of 200 pregnant women 7% had non-specific positive results to the VDRL test and 9·6% non-specific results to the FTA-ABS test. On the other hand, Manikowska-Lesińska[10] found that of 2000 pregnant women 0·2% had non-specific results to the FTA-ABS test; this is 2·5 times fewer than our percentage for the non-absorbent FTA test (1·65%). This indicates the value of the absorption method in routine diagnosis. It is curious that fewer false-positive results were obtained with the cardiolipin tests than with the FTA-ABS test.[9 21]

Thus the FTA and FTA-ABS tests, which were originally believed to be quick, simple, and reliable methods of confirming cardiolipin test results, have created unexpected additional problems and doubts. It should be remembered however that at the same time they usually readily demonstrate false-positive cardiolipin results. Of 3017 patients 0·66% had doubtful results after the routine use of the FTA-ABS test which required additional confirmation. In addition, we discovered 11 cases of late syphilis.

References

1. Moore JE, Lutz WB. The natural history of systemic lupus erythematosus, an approach to its study through chronic biologic false-positive reactors. *J Chronic Dis* 1955; **1**: 297-316.
2. Garner MF, Backhouse JL. Chronic biological false-positive reaction to serological test for syphilis in blood donors. *J Clin Pathol* 1970; **23**: 478-80.
3. Beal RW, Merry DJ. VDRL test in a blood transfusion service. *J Clin Pathol* 1975; **28**: 854-9.
4. Pien FD, Markowitz H, McKenna CH, Schroeter AL. Problems with beaded fluorescence pattern in FTA-ABS test. *J Am Vener Dis Assoc* 1976; **3**: 20-4.
5. Schneller WA, Izuno GT. Immunizations and false-positive VDRL test in recruits. *Milit Med* 1976; **141**: 93-6.
6. Catterall RD. Systemic disease and the biological false-positive reaction. *Br J Vener Dis* 1972; **48**: 1-12.
7. Clair RA, Montagne TS, Keetin LM. Biological *Treponema pallidum* immobilization test. *J Med* 1972; **3**: 264-9.
8. Meyer-Rohn J. Unspecifisch positive reaktionen in FTA test. *Hautarzt* 1974; **6**: 528-9.
9. Sarandria JL. False-positive FTA-ABS in pregnancy. *J Am Vener Dis Assoc* 1975; **1**: 165-8.
10. Manikowska-Lesińska W, Linda B, Zajac W. Specificity of the FTA-ABS and TPHA tests during pregnancy. *Br J Vener Dis* 1978; **54**: 295-8.
11. Miller JL, Brodley M, Hill JH. Studies on significance of biologic false-positive reaction. *JAMA* 1957; **13**: 1461-5.
12. Strejček J, Malina L, Bielicky T. Antinuclear factors, rheumatoid factors and Bordet-Wassermann reaction in chronic and systemic lupus erythematosus. *Acta Derm Venereol* 1968; **48**: 198-202.
13. Johansson EA. The use of standard serological tests for syphilis in screening for auto-immune connective tissue disease. *Acta Derm Venereol* 1970; **50**: 305-8.
14. Kraus SJ, Haserick JR, Lantz MA. Fluorescent treponemal antibody-absoption test reaction in lupus erythematosus. A typical beading pattern and probable false-positive reactions. *N Engl J Med* 1970; **282**: 1287-90.
15. Gibowski M, Wójtowicz M, Silny W, Karpisiewicz A. Biological false-positive syphilitic reaction on the material from the Clinical Hospital No. 2 Medical Academy in Poznan *Pol Tyg Lek* 1976; **41**: 1763-5.
16. Shore RN, Faricelli JA. Borderline and reactive FTA-ABS results in lupus erythematosus. *Arch Dermatol* 1977; **113**: 37-40.
17. Scotti AT, Mackey DM, Trautman JR. Syphilis and biological false-positive reactors among leprosy patients. *Arch Dermatol* 1970; **101**: 328-30.
18. Lesiński J, Miedziński F, Towpik J. *Współczesna Syfilidologia*. 1st ed. Warszawa: Państwowy Zakład Wydawnictw Lekarskich, 1970.
19. Wright JT, Cremer AW, Ridgway GL. False-positive FTA-ABS results in patients with genital herpes. *Br J Vener Dis* 1975; **51**: 329-32.
20. Chapel T, Jeffries CD, Brown WJ, Stewart JA. Influence of genital herpes on results of fluorescent treponemal antibody-absorption test. *Br J Vener Dis* 1978; **54**: 299-302.
21. Dans PE, Judson FN, Larsen SA, Lantz MA. The FTA-ABS test a diagnostic help or hindrance? *South Med J* 1977; **70**: 312-5.

British Journal of Venereal Diseases, 1980, **56**, 20-25

Antitreponemal IgE in early syphilis

J D BOS, F HAMERLINCK, AND R H CORMANE

From the Department of Dermatology, University of Amsterdam, The Netherlands

SUMMARY Using a solid-phase radioimmunoassay technique, mean serum IgE concentrations were found to be raised in patients with early syphilis. Antitreponemal specificity of the IgE response was investigated by the fluorescent treponemal antibody absorption test using a fluorescein-isothiocyanate-labelled antiserum against the Fc-fragment of human IgE. Validity of this test procedure was assessed by blocking experiments.

The results provide evidence of the antitreponemal specificity of the IgE response in syphilis and indicate a possible role for antitreponemal IgE in the pathogenesis of the Jarisch-Herxheimer reaction and in the immune-complex origin of some of the lesions of secondary syphilis.

Introduction

Antibodies of the different immunoglobulin classes can be separately detected by the use of monospecific antihuman fluorescein-isothiocyanate-(FITC)-labelled immunoglobulins. The use of monospecific antisera against human IgG, IgA, and IgM to detect antibodies using the fluorescent treponemal antibody (FTA) test have been reported by several authors.[1-5] These reports have indicated specific antitreponemal IgG, IgA, and IgM in sera from patients with syphilis. Using the FTA-200 test, Matuhasi *et al*[6] could not demonstrate IgD antibodies.

No reports on the existence of an IgE antitreponemal antibody have been published. Green *et al*[7] however found that the mean serum IgE concentrations for groups of healthy controls was significantly lower than that for a group of patients with primary syphilis.

In this study IgE concentrations in syphilis were investigated and the antitreponemal specificity of the IgE response evaluated.

Methods

The FTA-ABS test was performed according to the standard method of Kawamura *et al*[8] with the modifications described below.

ANTIGEN PREPARATIONS

Marked slides (see below) containing eight circles of 0·6 cm diameter were stored in 96% alcohol. Fresh *Treponema pallidum* suspension (see Materials) was thoroughly mixed and centrifuged at 200 × *g* for 10 minutes to free the suspension of cellular (rabbit) debris. The suspension was then diluted with distilled water until 30-40 micro-organisms per field were visible at × 400 magnification by darkground microscopy and allowed to mix for 10 minutes. Before use, these slides were wiped with absorbent paper. On each circle, 0·01 ml of the diluted antigen suspension was applied and allowed to dry at 37°C for 60-90 minutes. After drying the preparations were fixed in acetone for 10 minutes and stored in polyethylene sealed phials at −70°C until required.

TEST SERA

Ten millilitres of peripheral blood was collected aseptically and allowed to clot for one hour at room temperature; it was then centrifuged for 10 minutes at 400 × *g*. The serum was pipetted off and divided into 0·2-ml aliquots. The sera were stored at −70°C until required. No inactivation was carried out. Before use the test sera were diluted in sorbent (see Materials) and incubated for 30 minutes at room temperature.

INCUBATION OF TEST SAMPLES

From each test sample (patient serum diluted 1/5 in sorbent, control serum diluted 1/5 in sorbent, patient serum diluted in phosphate-buffered saline (PBS) for estimation of titre), 0·02 ml was applied to the antigen circle and incubated in a humid atmosphere at 37°C for 30 minutes. The slides were then gently

Address for reprints: Dr J D Bos, Department of Dermatology, University of Amsterdam, Binnengasthuis, Amsterdam, The Netherlands

Received for publication 10 May 1979

washed with PBS and placed in dishes containing PBS for 10 minutes.

INCUBATION OF CONJUGATES

After the slides had dried, 0·005 ml of PBS-diluted conjugate (see Materials) was applied to each antigen smear and incubated in a humid atmosphere for 30 minutes at 37°C. The experiments were ordered in such a way that only one type of conjugate was used for each antigen slide. After incubation, washing and drying were carried out as for the test samples.

MICROSCOPY

Mounting fluid and cover slips were applied to the slides after they had dried. A Leitz Orthoplan microscope with a Xenon XBO-75 lamp and a Leitz vertical illuminator was used. FITC-fluorescence was detected with the filter combination BG 38 (4 mm), KP 490 (2 mm), GG 475 (2 mm), and a secondary filter OG 513 (3 mm). For routine use examination was performed with a × 10 ocular and a Leitz × 40 dry objective. The results were read and interpreted as follows: strong fluorescence, positive (+); weak fluorescence, borderline (+/−); and no fluorescence, negative (−).

CONTROLS

In each experiment, the following controls were always included for each of the applied monospecific antisera: (1) positive control serum (see Materials) diluted 1/5 in sorbent; (2) negative control serum (see Materials) diluted 1/5 in sorbent; (3) sorbent only; and (4) antigen and conjugate but no human serum to serve as a control for non-specific staining of the antigen. Since the sorbent used during this study was from one manufacturer's batch tests to evaluate its absorbing activity were done infrequently.

FITC-LABELLED ANTIHUMAN GLOBULIN

For research purposes antihuman FITC-labelled monospecific conjugates directed against human Ig, Fc-fragments of human IgG, IgA, IgM, IgD, and IgE were used. Specificity of the antisera was demonstrated in direct cytoplasmic immunofluorescent staining of monoclonal human bone marrow cells and in immunoelectrophoresis.

For each conjugate, a chess-board titration against the positive reference serum was carried out to indicate the working dilution (Tables I and II). As non-specific fluorescence occurred the conjugate was absorbed with a suspension of *T pallidum*.

TABLE II *Results of limited chess-board titrations of GAHu/IgE(Fc)/FITC after previous absorption with T pallidum suspension and of two other unrelated antihuman IgE conjugates*

	Control sera			
Antiserum dilution	*Non-specific*	*Sorbent*	*Negative (1/5)*	*Positive (1/5)*
GAHu/IgE(Fc)/FITC				
1/20	−	−	−	+
1/40	−	−	−	+
1/80	−	−	−	−
1/160	−	−	−	−
GAHu/IgE/FITC				
1/20	−	−	−	+
1/40	−	−	−	+/−
1/80	−	−	−	+/−
1/160	−	−	−	−
ShAHu/IgE/FITC				
1/20	−	−	−	−
1/40	−	−	−	−
1/80	−	−	−	−
1/160	−	−	−	−

GAHu = goat antihuman conjugate
ShAHu = sheep antihuman conjugate
+ Positive, strong fluorescence; +/− borderline, weak fluorescence; − negative, no fluorescence

TABLE I *Results of chess-board titrations of GAHu/IgE(Fc)/FITC without previous absorption with a Treponema pallidum suspension*

	Control sera				*Positive reference control serum dilutions*							
Anti-serum dilutions	*Non-specific*	*Sorbent only*	*Negative (1/5 dilution)*	*Positive (1/5 dilution)*	*1/5*	*1/20*	*1/50*	*1/100*	*1/200*	*1/400*	*1/800*	*1/1600*
1/1					+	+	+	+	+	+	+	+
1/10					+	+	+	+	+	+	+	+
1/20	+/−	+	+	+	+	+	+	+	+	+	+/−	+/−
1/40	−	−	−	+	+	+	+	+	+/−	−	−	−
1/80	−	−	−	+/−	+	+/−	+/−	−	−	−	−	−
1/160	−	−	−	−	−	−	−	−	−	−	−	−
1/320					−	−	−	−	−	−	−	−
1/640					−	−	−	−	−	−	−	−

+ Positive, strong fluorescence; +/− borderline, weak fluorescence; − negative, no fluorescence

P 25,895

T PALLIDUM/CONJUGATE ABSORPTION PROCEDURE

Dilutions of a *T pallidum* suspension (Baltimore Biologic Laboratories (BBL), Cockeysville, Maryland) were made with PBS. The exact proportions of antiserum and *T pallidum* suspension were determined in previous titrations.

Five microlitres of a 1/100 dilution of *T pallidum* suspension were added to 500 μl of goat antihuman (GAHu)/IgE(Fc)/FITC conjugate and mixed. The *T pallidum*/conjugate mixture was incubated for one hour at 37°C and centrifuged for one hour at 12 000 rev/min in a cold environment (4°C); supernatants were divided into small aliquots and stored at −20°C until required.

DETERMINATION OF IgE CONCENTRATIONS

Total IgE concentrations of a group of IgE-FTA-ABS-test-positive sera were determined by a non-competitive solid-phase radioimmunoassay[9] at the Central Laboratory of the Red Cross Blood Transfusion Services (Amsterdam, The Netherlands). IgE concentrations of the control group were also made available by the Central Laboratory.

SERUM SPECIMENS

Consecutive serum specimens from the venereology outpatient department of our hospital were investigated by all monospecific FTA-ABS tests. Sera from patients with definite allergic symptoms or signs were excluded from the statistical analysis.

Determinations of antitreponemal IgE titres in the FTA test were performed in PBS dilutions without previous absorption.

STATISTICAL METHODS

The χ^2 test (with Yates's correction) and Student's *t* test were used for statistical analysis of IgE concentrations.

SPECIFICITY TESTS

Blocking experiments

To ascertain the specificity of the observed fluorescence in the IgE-FTA-ABS test, a blocking experiment was performed. In this procedure unlabelled and diluted antisera* against the different immunoglobulin classes were incubated on antigen slides after IgE-positive sera had been applied but before incubation with the FITC-labelled anti-IgE in the working dilution of 1/20 in PBS. Blocking—that is, preventing the occurrence of otherwise expected fluorescence—may only be obtained with unlabelled anti-IgE.

Three known IgE-FTA-ABS-test-positive sera, obtained from patients with primary, secondary, and early latent syphilis, were included in the assay. Unlabelled swine antihuman antisera against IgG, IgA, IgM, and IgE were used as blocking agents.

Use of other FITC-labelled antihuman IgE conjugates

Another way of proving the specificity of the test procedure is to use other unrelated commercially available FITC-labelled antihuman IgE conjugates. Limited chess-board titrations were therefore carried out with FITC-labelled antihuman IgE antisera obtained from distinct sources.

Materials

ANTIGEN

T pallidum (Nichols strain) was used as antigen. A fresh *T pallidum* suspension was provided by the National Institute of Public Health, Bilthoven, The Netherlands, and transported in Nelson's medium. For absorption of non-specific fluorescence from the anti-IgD and IgE conjugates, a *T pallidum* suspension obtained from BBL (batch No L9ELVP) was used.

CONTROL SERA

A pooled positive control reference serum was also provided by the National Institute of Public Health. No decrease in fluorescence was observed when dilutions in sorbent were compared with dilutions in PBS. Non-specific and negative control sera were obtained from healthy, non-syphilitic volunteers.

SORBENT

A FTA-ABS test sorbent, prepared according to the standardised method described by Stout *et al*[10] (obtained from BBL) (batch No L9ELSW), was used.

HUMAN SYPHILITIC REFERENCE SERUM

A lyophilised international reference preparation of human syphilitic serum was obtained from the Statens Seruminstitut, Copenhagen, Denmark.

PHOSPHATE-BUFFERED SALINE (pH 7·4)

PBS (8·2 g NaCl + 1·6 g $Na_2HPO_4.2H_2O$ + 0·2 g $NaH_2PO_4.2H_2O$ dissolved in 1 litre distilled water) was used.

SOLVENT

Reagent-grade acetone was used for fixation of slides.

OBJECT SLIDES

Toxoplasmosis slides (Bellco Glass Inc., Vineland, New Jersey) were used.

---

*Specifications and working dilutions of antisera used may be obtained from the authors.

MOUNTING FLUID

FA mounting fluid, pH 7·2, (Difco Laboratories, Detroit, Michigan) was used.

Results

MONOSPECIFIC FTA-ABS TESTS

From the results of chess-board titrations of all monospecific antihuman conjugates against the positive reference serum, it was concluded that syphilitic sera contained antitreponemal activity in the IgG, IgA, IgM, and, unexpectedly, IgE subclass of immunoglobulins (Tables I and II). No activity was noted in the IgD class.

SPECIFICITY TESTS

From the results of blocking experiments, unlabelled anti-IgE in a working dilution of 1/80 in PBS was able to prevent the occurrence of otherwise expected fluorescence (Table III). Unlabelled anti-IgG, anti-IgA, and anti-IgM were unable to prevent the occurrence of fluorescence at the same working dilution. From the results of limited chess-board titrations with other FITC-labelled anti-IgE sera (Table II) the GAHu/IgE(Fc)/FITC conjugate (obtained from Meloy Laboratories Inc) gave similar results whereas the sheep anti-IgE gave negative results. The latter observation is difficult to explain, but differences in staining properties between distinct conjugates is well-known in immunofluorescence.[11]

Thus results of specificity tests strongly support the specificity of the test procedure and confirm the existence of an antitreponemal IgE in syphilis.

IgE-FTA-ABS TEST

A total of 116 sera from patients attending the outpatient department were studied, together with sera from 20 healthy volunteers (Table IV).

TABLE IV *Results of IgE-FTA-ABS tests in 44 patients with syphilis, 47 patients with other conditions, and 45 healthy persons (expressed in absolute numbers)*

	IgE/FTA-ABS test result			
Diagnosis	+	+/−	−	*Total*
Primary syphilis	15	2	12	29
Secondary syphilis	6	0	3	9
Early latent syphilis	4	0	0	4
Tertiary syphilis	2	0	0	2
Total	27 (61%)	2 (5%)	15 (34%)	44
Ulcus molle	0	0	5	5
Herpes simplex (types 1 and 2)	0	0	13	13
Non-specific ulcer*	0	1	15	16
Gonorrhoea	0	0	5	5
Chlamydial urethritis	0	0	3	3
Acute biological false-positive reactor	0	0	5	5
Healthy controls	0	0	20	20
Healthy contacts†	0	0	25	25
Total	0	1	91	92
Total	27	3	106	136

*An ulcer which healed spontaneously and from which *T pallidum* could not be found by darkground microscopy and gave negative results to herpes virus culture, *H ducreyi* culture, and to serological tests and culture for lymphogranuloma venereum.

†Patients attending as contacts of syphilis but in whom there were no clinical or serological indications of infection after observations for six months.

+ Positive, strong fluorescence; +/− borderline, weak fluorescence; − negative, no fluorescence.

Although the numbers were small, the results of the IgE-FTA-ABS tests indicate an increase in antitreponemal IgE with longer duration of antigenic stimulation with *T pallidum*. When the results of the FTA-ABS test with anti-IgG, anti-IgM, and anti-IgE were compared in individual sera obtained at various intervals after infection it became evident that the already well-known primary IgM response against *T pallidum* was followed by an antitreponemal IgE and IgG response.

IgE CONCENTRATIONS IN POSITIVE TEST SERA

A total of 19 sera were obtained and investigated, all from patients in the early stages of syphilis. In the patients with early syphilis 13 (68%) of 19 sera contained more than 100 IU/ml of IgE whereas in the

TABLE III *Blocking of FITC-labelled antihuman IgE with unlabelled antihuman IgE but not with unlabelled antihuman IgG, IgA, or IgM (1/80 PBS)*

		Specificity and working dilution of unlabelled antisera							
		IgG		*IgA*		*IgM*		*IgE*	
Serum No	*Dilution (1/5)*	*1/40*	*1/80*	*1/40*	*1/80*	*1/40*	*1/80*	*1/40*	*1/80*
78-13	PBS	+	+	+	+	+	+	+/−	−
	Sorbent	+	+	−	+	+	+	−	−
78-27	PBS	−	+	+	+	+	+	+/−	−
	Sorbent	−	+	+	+	−	+	−	−
78-58	PBS	−	+	+	+	+	+	−	−
	Sorbent	−	+	+	+	+	+	−	−

PBS = phosphate-buffered saline

+ Positive, strong fluorescence; +/− borderline, weak fluorescence; − negative, no fluorescence healthy control group only eight (32%) of 25 sera contained more than 100 IU/ml. This difference is statistically significant (χ^2 $0\cdot025<P<0\cdot05$). (Precise numbers were given when the results were above 100 IU/ml whereas in cases where the result was below 100 IU/ml no further specification was obtained.) IgE-FTA-ABS test positive sera from patients from tropical or subtropical countries were excluded from the statistical analysis.

Furthermore, the mean IgE concentration from patients with primary and secondary syphilis above 100 IU/ml (332 IU/ml; range 100-680 IU/ml) was significantly higher than that in the healthy control group (204 IU/ml; range 110-370 IU/ml) ($t=2\cdot0$, $0\cdot025<P<0\cdot05$).

From these data infection with *T pallidum* appears to be associated with an increase in serum IgE concentration.

ESTIMATION OF IgE TITRES

End-point titrations of IgE-positive sera were carried out in PBS-dilutions without previous absorption of the sera with *Treponema phagedenis* (Reiter) sorbent. The range of antitreponemal IgE titres was 50-1600 with a mean value of 340 (reciprocal value). No correlation occurred with total IgE concentrations.

Discussion

The results of determinations of IgE concentrations support the findings of Green *et al*,[7] although in our study IgE concentrations in patients with primary and secondary syphilis were evaluated as a group without further differentiation of the stage of disease. Taken together, these studies indicate an IgE response in *T pallidum* infection.

The association between immunodeficiencies, especially T-lymphoid cell deficiency, and hyper-IgE production is well known,[12] and increased IgE production may be related to decreased cellular immunity in the early stages of the disease.[3] In fact studies presently being carried out in our laboratory indicate decreased numbers of circulating T-lymphoid cells as determined by the rosette technique.

Performance of the IgE-FTA-ABS test and results of analysis of its specificity provide substantial evidence for the antitreponemal specificity of the IgE response. The separation of IgG and IgE in monospecific FTA-ABS tests results indicate that on antigenic stimulation of the host with *T pallidum* firstly an IgM response is initiated followed by the simultaneous production of anti-treponemal IgG and IgE.

Possibly antitreponemal IgE plays a part in the pathogenesis of the Jarisch-Herxheimer reaction. In this reaction, a complex series of clinical and physiological events follows the start of treatment in patients with syphilis and in other spirochaetoses, such as yaws and louse-borne relapsing fever.[14] In primary seronegative syphilis the incidence of the reaction was estimated to be 55% whereas in primary seropositive syphilis the incidence can rise to 95%.[15] Degranulation of mast cells and basophil leucocytes on binding of treponemal antigens with membrane-bound IgE with a subsequent release of histamine, slow-reacting substance of anaphylaxis (SRS-A), chemotactic factor for eosinophil leucocytes, and platelet aggregating factor may account for at least some of the symptoms and signs of the Jarisch-Herxheimer reaction.

Another clinical aspect of antitreponemal IgE is its possible role in the development of immune-complex disease, which may be of importance in the aetiology of some of the lesions in secondary syphilis.[16] Platelet aggregating factor may induce platelet accumulation at the endothelial site followed by release of platelet mediators; these increase vascular permeability, and circulating immune complexes may be deposited in the vessel wall damaging tissue.[17]

Finally it should be pointed out that fluorescence in the IgE-FTA-ABS test is considerably less than in the IgG-FTA-ABS test, thus making the procedure unsatisfactory for routine use. Its results however may be helpful in understanding the pathogenesis of syphilis.

These investigations were made possible by a grant from the Prevention Fund (The Hague, The Netherlands). We are also much indebted for the technical advice and support of Dr R C Aalberse, Dr H E Menke, and J J Snooijink.

References

1. Julian AJ, Logan LC, Norins LC. Early syphilis: immunoglobulins reactive in immunofluorescence and other serologic tests. *J Immunol* 1969; **102:** 1250-9.
2. Manikowska-Lesinska W, Jakubowski A. Relationship of antibodies detected by the immunofluorescence method with the various classes of immunoglobulins in cases of untreated syphilis. *Br J Vener Dis* 1970; **46:** 380-2.
3. O'Neill P, Nicol CS. IgM-class antitreponemal antibody in treated and untreated syphilis. *Br J Vener Dis* 1972; **48:** 460-3.
4. Müller F, Loa PL. Neue möglichkeiten in der immunologischen diagnostik der treponemen-infektion (syphilis). *Infection* 1974; **2:** 127-31.
5. Wilkinson AE, Rodin P. IgM-FTA test in syphilis in adults: its relation to clinical findings. *Br J Vener Dis* 1976; **52:** 219-23.
6. Matuhasi T, Mizuoka K, Usui N. Studies on the fluorescent treponemal antibody test using fluorescent anti-γ, anti-α, anti-μ, anti-x, anti-λ chains and βICA, E globulin reagents. *Bull WHO,* 1966; **34:** 466-72.
7. Green RL, Scales RW, Kraus SJ. Increased immunoglobulin E concentrations in venereal diseases. *Br J Vener Dis* 1976; **52:** 257-60.

8. Kawamura A, Misuoka K, Matuhasi T, Fukuoka Y. Methods used in Japan for FTA-ABS tests (fluorescent treponemal antibody test) In: EH Beutner *et al*, eds. Stroudsberg, Pennsylvania: *Immunopathology of the Skin: Labeled Antibody Studies*. Dowden Hutchinson and Ross, 1973: 425-45.
9. Stallman PJ, Aalberse RC. Estimation of basophil-bound IgE by quantitative immunofluorescence microscopy. *Int Arch Allergy Appl Immunol* 1977; **54**: 9-18.
10. Stout GW, Kellogg DS, Jr, Falcone VH, McGrew BE, Lewis JS. Preparation and standardization of the sorbent used in the fluorescent treponemal antibody-absorption test. *Health Lab Sci* 1967; 4: 5-8.
11. Cormane RH, Holubar K. Immunofluorescence staining properties of different conjugates. *Dermatologica* 1969; **139**: 134-44.
12. Strannegård Ö, Strannegård I-L. T-lymphocyte numbers and function in IgE-mediated allergy. *Immunol Rev* 1978; **41**: 149-70.
13. Pavia CS, Folds JD, Baseman JB. Cell-mediated immunity during syphilis. *Br J Vener Dis* 1978; **54**: 144-50.
14. Fulford KWM, Johnson N, Loveday C, Storey J, Tedder RS. Changes in intravascular complement and antitreponemal antibody titers preceding the Jarisch-Herxheimer reaction in secondary syphilis. *Clin Exp Immunol* 1976; **24**: 483-91.
15. Putkonen T, Salo OP, Mustakallio KK. Febrile Herxheimer reaction in different phases of primary and secondary syphilis. *Br J Vener Dis* 1966; **42**: 181-4.
16. Sølling J, Sølling K, Jakobsen KU, From E. Circulating immune complexes in syphilis. *Acta Derm Venereol (Stock)* 1978; **58**: 263-7.
17. Benveniste J. Definition expérimentale d'un rôle nouveau pour l'IgE: Déclenchement immunologique de la déposition des immuns complexes. *Nouv Presse Méd* 1973; **2**: 703-6.

British Journal of Venereal Diseases, 1980, **56,** 26-30

Auxotypes and antimicrobial susceptibilities of *Neisseria gonorrhoeae* in black and white patients

ROBERT C NOBLE AND BETTY R MILLER
From the Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky, USA

SUMMARY Isolates of *Neisseria gonorrhoeae* from 100 white and 113 black patients attending a venereal disease clinic were examined for their susceptibility to ampicillin, penicillin, tetracycline, and spectinomycin. The isolates were also characterised by gonococcal auxotyping. Gonococcal isolates from black patients were more resistant to the antibiotics than those from white patients, since the former were infected with the more antibiotic-resistant auxotypes (Pro, Zero, and Arg) whereas the latter were infected with the more antibiotic-susceptible auxotypes (AHU and others). These data indicate a preferential infection by sub-populations of *Neisseria gonorrhoeae* in two racial groups.

Introduction

Despite the availability of effective antimicrobial therapy, gonococcal infections continue to be a major public health problem. Epidemiological investigations have shown that gonorrhoea occurs more commonly in black patients than in white patients attending venereal disease clinics in the United States.[1-3] It is not known if this is due to behavioural, genetic, or some other biological phenomenon. One possible explanation is that gonococci infecting black and white patients differ in certain critical properties that would be disadvantageous to black individuals, such as the susceptibility of the gonococci to antibiotics used in treatment. Patients who are infected with gonococci that are relatively resistant to certain antibiotics have a higher treatment failure rate than individuals infected with less resistant isolates.[4] Such inadequately treated patients may remain infectious and continue to contribute to the total number of cases of gonorrhoea. If antibiotic-resistant gonococci occurred more frequently in black patients, this might partly explain the increased incidence of gonorrhoea in black populations.

Our study was designed to examine gonococci isolated from black and white patients attending a venereal disease clinic and to test the susceptibility of the gonococci to antimicrobial agents used in treatment. The gonococci were further characterised by auxotyping.[5]

Patients and methods

BACTERIAL ISOLATES

Isolates of *Neisseria gonorrhoeae* were cultured from 213 patients with uncomplicated gonorrhoea (138 men, 75 women) attending the Lexington-Fayette County Health Department between December 1973 and December 1977. At the time of registration in the clinic, the patients completed an admission form which included a designation of race as either white or non-white. Black patients accounted for almost all the patients in the non-white category. Clinic attendances by Indians, Orientals, or other racial groups were rare because of the geographical location of the clinic.

Only one isolate was studied from a single patient and the culture sites included urethra, cervix, or rectum. All isolates were obtained before antibiotic therapy. The cultures were taken with cotton swabs and incubated for 48 hours at 36°C on modified Thayer-Martin medium containing vancomycin, colistin, nystatin, and trimethoprim.[6] All gonococci were identified by bacterial morphology, Gram stain, oxidase reaction (1% solution of N, N-dimethyl-p-phenylenediamine monohydrochloride), and the production of acid with dextrose- but not maltose-, lactose-, sucrose-, or fructose-containing media.

Address for reprints: Dr R C Noble, Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky 40536, USA

Received for publication 12 April 1979

Gonococcal isolates were stored at −70°C in tryptic soy broth containing 13% glycerol.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic susceptibility testing was performed using the plate dilution technique.[4] Strains of *N gonorrhoeae* (F18 and F29) and *Sarcinia lutea* (F20) with known antibiotic susceptibilities (provided by Dr Clyde Thornsberry, Center for Disease Control, Atlanta, Georgia) were included as controls when gonococcal isolates were tested for their antibiotic susceptibilities. The antibiotics (purchased from the United States Pharmacopeial Convention Inc., Bethesda, Maryland) were tested in the following concentrations by means of two-fold dilutions in agar: ampicillin and penicillin 0·007-2 μg/l; tetracycline 0·09-25 μg/l; and spectinomycin 0·39-100 μg/l.

AUXOTYPING

Gonococcal auxotyping was performed on each isolate, and gonococci of known type were included with each batch. Single isolates of four different auxotypes (Zero, Thi, Pro-Hyx, Pro-Met-ThPP) (supplied by Dr Karen Carifo and Dr B Wesley Catlin, Medical College of Wisconsin) were used.

STATISTICAL METHODS

The χ^2 test was used in the analysis of categorical data. The Wilcoxin-Mann-Whitney U test (two-tailed) and the Kruskal-Wallis one-way analysis of variance were used in the analysis of the antibiotic susceptibility data.[7] The second and third tests included corrections for tied observations.

Results

One hundred and thirteen patients identified themselves as black and 100 patients as white. Gonococcal isolates from black patients were significantly more resistant to ampicillin, penicillin, tetracycline, and spectinomycin than those from the white patients (Table I). The percentages of the auxotypes of gonococci isolated from the black and white patients are shown in Table II. The auxotypes of the gonococci differed significantly between the racial groups. Gonococci of the auxotypes, Pro, Zero, and Arg, were isolated more frequently from black patients whereas gonococci of the auxotypes, Arg-Hyx-Ura and "others", were isolated more frequently from whites. The latter category contained the following auxotypes; two Pro-Arg, one Pro-Met, five Pro-Arg-Hyx-Ura, one Arg-Met-Hyx-Ura, one Arg-Hyx-Pro, three Arg-Orn-Hyx-Ura, and two Arg-Orn. In addition, gonococci of the five auxotype groups differed in their susceptibility to the four antibiotics (Table III). The gonococci of auxotypes, Pro, Zero, and Arg, were more resistant to the antibiotics whereas those of the Arg-Hyx-Ura and other auxotypes were more susceptible.

Within any one of the auxotype groups, there was no significant difference in the antimicrobial susceptibilities of the gonococcal isolates from black and white patients. Also there was no significant association between the site from which the gonococci were cultured—that is, the cervix, rectum, and urethra—and their auxotype or their susceptibility to the four antibiotics.

TABLE I *Susceptibility to four antibiotics of Neisseria gonorrhoeae isolates from black and white patients*

Race	*% of total No of isolates susceptible to:*									*Total*
Ampicillin (μg/ml)	*0·007*	*0·015*	*0·03*	*0·06*	*0·125*	*0·25*	*0·5*	*1·0*	*2·0*	
White	3·4	27·3	22·7	9·1	8·0	15·9	12·5	1·1	0	88
Black	0·9	1·8	6·2	9·8	24·1	24·1	31·2	1·8	0	112
	Z=4·94	P<0·00003								
Penicillin (μg/ml)	*0·007*	*0·015*	*0·03*	*0·06*	*0·125*	*0·25*	*0·5*	*1·0*	*2·0*	
White	29·6	20·4	9·2	2·0	10·2	11·2	9·2	7·1	1·0	98
Black	3·6	7·1	4·5	9·8	19·6	17·9	24·1	10·7	2·7	112
	Z=5·66	P<0·00003								
Tetracycline (μg/ml)	*0·09*	*0·19*	*0·39*	*0·78*	*1·56*	*3·13*	*6·25*	*12·5*	*25*	
White	27·2	38·3	12·8	16·0	4·3	1·1	0	0	0	94
Black	9·0	36·9	18·0	26·1	9·0	0	0	0·9	0	111
	Z=3·54	P<0·0003								
Spectinomycin (μg/ml)			*0·39*	*0·78*	*1·56*	*3·13*	*6·2*	*12·5*	*25*	
White			1·0	1·0	3·1	10·4	36·5	46·9	1·0	96
Black			0	0	0	4·5	26·8	67·9	0·9	112
	Z=3·30	P<0·0005								

*Normal deviate Z according to the normal approximation for the Wilcoxin-Mann-Whitney U test (two-tailed)

TABLE II *Auxotypes of gonococcal isolates from black and white patients*

Race	*Auxotypes (% of total No of isolates)* *Zero*	*Pro*	*Arg*	*Arg-Hyx-Ura*	*Other*	*Total No*
White	22·0	25·0	4·0	35·0	14·0	100
Black	30·1	54·9	11·5	2·7	0·9	113

$\chi^2_4 = 60\cdot79$; P<0·001

TABLE III *Susceptibility to four antibiotics of auxotypes of Neisseria gonorrhoeae*

Auxotype	*% of total No of isolates susceptible to:*									*Total No*
Ampicillin (μg/ml)	*0·007*	*0·015*	*0·03*	*0·06*	*0·125*	*0·25*	*0·5*	*1·0*	*2·0*	
Zero	1·8	7·3	7·3	23·6	18·2	29·1	12·7	0	0	55
Pro	0	0	6·1	6·1	26·8	22·0	36·6	2·4	0	82
Arg	0	0	6·7	0	0	40·0	53·3	0	0	15
Arg-Hyx-Ura	3·0	51·5	39·4	0	6·1	0	0	0	0	33
Other	13·3	33·3	26·7	6·7	0	6·7	6·7	6·7	0	15
	H* = 89·28	df = 4	P<0·001							
Penicillin (μg/ml)	*0·007*	*0·015*	*0·03*	*0·06*	*0·125*	*0·25*	*0·5*	*1·0*	*2·0*	
Zero	7·4	7·4	13·0	7·4	33·3	20·3	9·3	1·8	0	54
Pro	1·1	5·8	3·5	9·3	15·1	17·4	27·9	16·3	3·5	86
Arg	11·8	0	0	0	5·9	23·5	41·2	17·6	0	17
Arg-Hyx-Ura	47·4	42·1	10·5	0	0	0	0	0	0	38
Other	53·3	20·0	0	6·7	0	6·7	0	6·7	6·7	15
	H = 93·84	df = 4	P<0·001							
Tetracycline (μg/ml)	*0·09*	*0·19*	*0·39*	*0·78*	*1·56*	*3·13*	*6·25*	*12·5*	*25*	
Zero	21·1	34·6	25·0	13·5	1·9	1·9	0	1·9	0	52
Pro	1·1	42·5	11·5	33·3	11·5	0	0	0	0	87
Arg	0	6·2	43·8	43·8	6·5	0	0	0	0	16
Arg-Hyx-Ura	50·0	47·2	2·8	0	0	0	0	0	0	36
Other	42·9	28·6	7·1	7·1	14·3	0	0	0	0	14
	H = 59·18	df = 4	P<0·001							
Spectinomycin (μg/ml)	*0·09*	*0·19*	*0·39*	*0·78*	*1·56*	*3·13*	*6·25*	*12·5*	*25*	
Zero	0	0	0	0	0	9·4	35·8	54·7	0	53
Pro	0	0	0	1·1	0	2·3	26·4	67·8	2·3	87
Arg	0	0	0	0	0	0	25·0	75·0	0	16
Arg-Hyx-Ura	0	0	2·6	0	7·9	18·4	31·6	39·5	0	38
Other	0	0	0	0	0	7·1	50·0	42·9	0	14
	H = 19·16	df = 4	P<0·001							

*According to the χ^2 approximation to the Kruskal-Wallis H test

Discussion

The auxotypes of gonococci infecting black patients differed from those infecting white patients in our clinic. Gonococci of the Pro, Zero, and Arg auxotypes were more common in black patients whereas those of the Arg-Hyx-Ura (AHU) and others were more common in white patients. Pro was the most common auxotype in blacks and AHU was the most common auxotype in whites. Our findings are consistent with those indicating that gonococci of the AHU auxotype are more common in white than black patients attending venereal disease clinics in the United States.[8] [9] Auxotypes of gonococci from our clinic and from Sweden and the Philippines are compared in Table IV. The AHU auxotype accounted for most of the isolates from Sweden[10] but

TABLE IV *Auxotypes of gonococci isolated from patients in different geographical areas*

Auxotype	*% of strains in each geographical area* *Lexington*	*Sweden*[10]	*Philippines*[11]
Zero	26·3	15·7	60·2
Pro	40·8	12·7	38·5
Arg	8·0	1·9	0
AHU	17·8	40·1	0
Other	7·0	29·6	1·3
Total No	213	166	78

was absent in the isolates from the Philippines, where the Zero and Pro auxotypes were more common.[11] Although the racial distribution of the patients was not given in these two studies, it is likely that gonococci from the Philippines originated from predominantly non-white patients and the gonococci from Sweden were cultured from predominantly white patients. Thus, the race of the patient may be a factor in the geographical variation of gonococcal auxotypes.

Gonococci of the Pro, Zero, and Arg auxotypes were less susceptible to the four antibiotics compared with those of the other auxotypes. Similar differences in the susceptibility to penicillin of gonococci of these auxotypes have been found by other workers.[8 9 11-14] Gonococci of the AHU auxotype are the most susceptible to penicillin and those of the Pro auxotype generally the most resistant to penicillin compared with those of the other auxotypes. If susceptibility to antibiotics is associated with certain gonococcal auxotypes, the changes in susceptibility of gonococci to antibiotics over a period of years may be the result of changes in the prevalence of particular auxotypes. For example, the increased prevalence of gonococci susceptible to penicillin in North Carolina from 1973 to 1978 could be accounted for by the increasing prevalence of AHU strains.[5] Also the presence of certain auxotypes may result in differences in the antibiotic susceptibility of gonococci in various geographical areas. In general, gonococci with relative penicillin resistance are uncommon in northern Europe but quite common in the Far East,[16] and this is consistent with the auxotyping data presented in Table IV.

We have not attempted in this study to examine behavioural, social, or genetic factors that might be responsible for the difference in the incidence of gonorrhoea or for the presence of different auxotypes in the two patient groups. In data from public venereal disease clinics, there is a possibility of bias in the selection of patients because of the unequal use of the facilities by blacks and whites.[2] Black men delay seeking treatment longer than white men after the onset of genitourinary symptoms.[17] If white men received treatment earlier for symptomatic infections, this might favour, in time, a higher proportion of AHU isolates in whites since this auxotype is more commonly associated with asymptomatic gonorrhoea in men.[8] However, this would not explain the relative rarity of this auxotype in blacks. If blacks received inadequate doses of antibiotics before their clinic visit and whites did not, this might also explain the low incidence of AHU isolates in blacks, since the AHU isolates are highly susceptible to antibiotics. However, in another study from our clinic performed at the same time as the present study, we found that the ingestion of antibiotics before the clinic visit was not associated with race.[3] Social segregation itself is an unlikely cause of microbiological segregation of gonococci, because there was considerable overlap in both groups in the auxotypes more commonly found in blacks. Possibly blacks have an increased susceptibility to gonorrhoea as the result of some genetic or other host factor. Racial differences in the colonisation by pathogenic bacteria have been found among patients for whom cultures for staphylococci and meningococci have been performed.[18-20] Colonisation by both these micro-organisms is more common in whites than in blacks contrary to what might be expected as a result of overcrowding or other factors associated with low socioeconomic status.

Genetic factors, such as the presence of certain blood-group antigens, may play a part in the increased susceptibility of patients to gonorrhoea. Black patients with blood group B had a higher incidence of gonorrhoea than black patients with other blood groups.[21] Although this observation was confirmed in white patients with blood group B, Miler *et al*[22] found no such correlation in black patients. Our definition of the two racial groups was based on self-declaration. Determination of histocompatibility antigens might also be useful in the description of this phenomenon. Preferential infection by subpopulations of gonococci in these two groups of patients has implications for the control of gonorrhoea. If antibiotic-resistant gonococci are isolated more frequently from black patients, efforts should be made to examine current treatment and test-of-cure regimens for this group.

We thank Dr Ernst Stolz, Department of Dermatology, and Dr R van Strik, Department of Biostatistics, Erasmus University, Rotterdam for their advice. We are also grateful to Ms A M Noble for her advice and assistance and to Ms Magda de Ridder-Goetjaer for typing the manuscript. This study was made possible through the co-operation of Philip G Weiler, Commissioner of Health, Lexington-Fayette County Health Department, and by grants from the National Institute of Allergy and Infectious Diseases (AI 11304) and from the Alexandrine and Alexander Sinsheimer Fund. We also thank the *Journal's* statistical referee for his time and effort.

References

1. Brooks GF, Darrow WW, Day JA. Repeated gonorrhea: an analysis of importance and risk factors. *J Infect Dis* 1978; **137:** 161-9.
2. Brown WJ. Trends and status of gonorrhea in the United States. *J Infect Dis* 1971; **123:** 682-8.

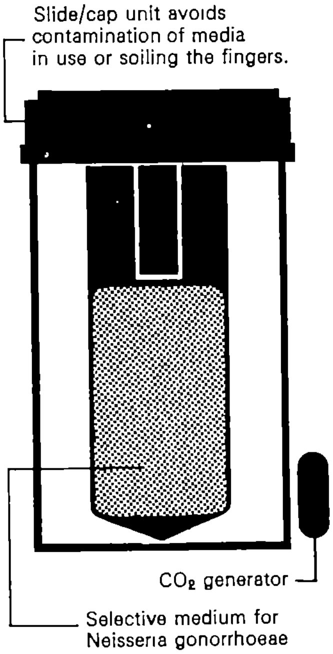
3. Noble RC, Kirk NM, Slagel WA, Vance BJ, Somes GW. Recidivism among patients with gonococcal infection presenting to a venereal disease clinic. *Sex Transm Dis* 1977; **4:** 39-43.
4. Jaffe HW, Biddle JW, Thornsberry C, *et al.* National gonorrhoea therapy monitoring study. In vitro antibiotic susceptibility and its correlation with treatment results. *N Engl J Med* 1976; **294:** 5-9.
5. Catlin BW. Nutritional profiles of *Neisseria gonorrhoeae, Neisseria meningitidis* and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. *J Infect Dis* 1973; **128:** 178-94.
6. Martin JE, Lester A. Transgrow a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis. Health Services and Mental Health Administration Health Reports* 1971; **86:** 30-3.
7. Siegel S. *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw Hill, 1956.
8. Crawford G, Knapp JS, Hale J, Holmes KK. Asymptomatic gonorrhea in men: caused by gonococci with unique nutritional requirements. *Science* 1977; **196:** 1352-3.
9. Knapp JS, Thornsberry C, Schoolnik GA, Wiesner PJ, Holmes KK, Co-operative Study Group. Phenotypic and epidemiologic correlates of auxotype in *Neisseria gonorrhoeae. J Infect Dis* 1978; **138:** 160-5.
10. Moberg I. Auxotyping of gonococcal isolates. In: Danielsson D, Julin L, Mårdh P-A, eds. *Genital Infections and their Complications.* Stockholm: Almqvist & Wiksell, 1975; 271-3.
11. Knapp JS, Holmes KK. Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. *J Infect Dis* 1975; **132:** 204-8.
12. Catlin BW, Pace PJ. Auxotypes and penicillin susceptibilities of *Neisseria gonorrhoeae* isolated from patients with gonorrhea involving two or more sites. *Antimicrob Agents Chemother* 1977; **12:** 147-56.
13. Morello JA, Lerner SA, Bohnhoff M. Characteristics of atypical *Neisseria gonorrhoeae* from disseminated and localized infections. *Infect Immun* 1976; **13:** 1510-16.
14. Schoolnik GK, Buchanan TM, Holmes, KK. Gonococci causing disseminated gonococcal infection are resistant to the bactericidal action of normal human sera. *J Clin Invest* 1976; **58:** 1163-73.
15. Eisenstein BI, Lee TJ, Sparling PF. Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated infection. *Infect Immun* 1977; **15:** 834-41.
16. Sparling PF. Antibiotic resistance in the gonococcus. In: Roberts RB, ed. *The gonococcus.* New York: John Wiley and Sons, 1977; 111-35.
17. Darrow WW. Venereal infections in three ethnic groups in Sacramento. *Am J Public Health* 1976; **66:** 446-50.
18. Millian SJ, Baldwin JN, Rheims MS, Weiser HH. Studies on the incidence of coagulase-positive staphylococci in a normal unconfined population. *Am J Public Health* 1960; **50:** 791-8.
19. Noble RC, Cooper RM, Miller BR. Pharyngeal colonisation by *Neisseria gonorrhoeae* and *Neisseria meningitidis* in black and white patients attending a venereal disease clinic. *Br J Vener Dis* 1979; **55:** 14-9.
20. Noble WC. Carriage of *Staphylococcus aureus* and beta hemolytic streptococci in relation to race. *Acta Derm Venereol (Stockh)* 1974; **54:** 403-5.
21. Foster MT, Labrum AH. Relation of infection with *Neisseria gonorrhoeae* to ABO blood groups. *J Infect Dis* 1976; **133:** 329-30.
22. Miler JJ, Novotny P, Walker PD, Harris JRW, MacLennan IPB. *Neisseria gonorrhoeae* and the ABO isohemagglutinins. *Infect Immun* 1977; **15:** 713-9.

NEW

Till~U~Test G.C. Slide

for simple culture of ***Neisseria gonorrhoeae***

- * **Incubation problems solved by self-contained unit with built-in anaerobic/CO_2 environment**
- * **No transport medium needed**
- * **Results in 24-48 hours**
- * **Selective culture medium for *N. gonorrhoeae***
- * **Economic in use.**


Slide/cap unit avoids contamination of media in use or soiling the fingers.
CO_2 generator
Selective medium for Neisseria gonorrhoeae

Further information from:

Tillomed Laboratories Ltd.
Henlow Trading Estate, Henlow, Beds.

79006

Penetrating Sites

Amoxil's ability to penetrate bronchial secretions whether purulent or mucoid, may explain why it has been shown to be more effective than cotrimoxazole or ampicillin in preventing relapse in bronchitis.[1] Amoxil also achieves significantly higher levels than ampicillin in both the maxillary sinus mucosa[2] and middle ear fluid.[3]

Powerful Spectrum

Amoxil's broad spectrum of activity extends across the Gram-positive and Gram-negative pathogens responsible for the vast majority of respiratory and urinary infections. In particular, Amoxil is notably active against H. influenzae, a predominating pathogen in lower respiratory infection and otitis media,[4] and against E. coli, the organism largely responsible for simple urinary infection in general practice.

Penicillin Safety

As a penicillin, Amoxil is well tolerated and suitable for most patients, including the elderly and pregnant women. Penicillin hypersensitivity is the only contra-indication.

Prescribing Simplicity

The absorption of Amoxil is virtually unaffected by the presence of food[5] which adds to the convenience of its tds dosage. And for urinary infections in adults, Amoxil Twin Pack, the complete two dose course of treatment, is simplicity itself.

Amoxil
amoxycillin

Proves Successful

Indications: Cystitis, urethritis, pyelonephritis. Bacteriuria in pregnancy. Gonorrhoea Skin and soft tissue infections. Acute and chronic bronchitis Upper respiratory tract infections. Otitis Media. Pneumonia **Presentations:** Amoxil capsules· 250mg and 500mg; PL 0038/0103/5 Amoxil syrup· 125mg and syrup forte 250mg per 5ml PL 0038/0108/9. Amoxil paediatric suspension: 125mg per 1.25ml, PL 0038/0107 Amoxil 3g sachet· 3g per 50ml; PL 0038/0238. Amoxil vials for injection. 250mg, 500mg and 1g, PL 0038/0221/2/6. The amoxycillin content per dose unit is present as the trihydrate in Amoxil oral preparations and as the sodium salt in Amoxil injections. Average daily cost for adults (250mg capsules t.d s.) is 35p and for children (125mg syrup t.d.s) is 24p at time of printing. **Dosage**· Adult Dosage Oral· 250mg three times a day. In severe infections doses should be doubled. In simple, acute urinary tract infection in adults two 3g doses amoxycillin (Twin Pack) with 10-12 hours between the doses. Injectable: 500mg IM 8 hourly (or more frequently if necessary) in moderate infections 1g IV 6 hourly in severe infections. **Childrens Dosage** (up to 10 years of age) Oral: 125mg three times a day. In severe infections doses should be doubled. Injectable: 50-100mg/kg bodyweight per day, in divided doses **Contra-indications:** Amoxil is a penicillin and should not be given to penicillin hypersensitive patients. **Side-effects:** Side-effects, as with other penicillins, are usually of a mild and transitory nature: they may include diarrhoea, indigestion or occasionally rash, either urticarial or erythematous. The urticarial rash suggests penicillin hypersensitivity, and the erythematous type rash may arise if Amoxil is administered to patients with glandular fever In either case treatment should be discontinued. Since Amoxil is a penicillin, problems of overdosage are unlikely to be encountered. Further information on Amoxil (regd) amoxycillin is available from·
Bencard, Great West Road, Brentford, Middlesex. Tel: 01-560 5151

References. 1. Chemotherapy (1977), 23, 58-64 2 Drugs (1975), 9, 81-140. 3. J Infect. Dis (1977), 135, 999-1002. 4. Brit. med. J (1976), 2, 1407 5. Brit med. J. (1972), 3, 13-16.

Bencard

13521

British Journal of Venereal Diseases, 1980, **56,** 31-34

Relationship of the interval between infections and the similarity of gonococcal strains in recurrent gonorrhoea

ROBERT C NOBLE, A P ORANJE, M F MICHEL, AND E STOLZ

From the Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky, USA, and the Departments of Dermatology and Clinical Microbiology and Antimicrobial Therapy, Erasmus University, Rotterdam, The Netherlands

SUMMARY Paired gonococcal isolates from 38 patients with recurrent episodes of gonorrhoea at varying intervals were examined for similarity by means of gonococcal auxotyping and susceptibility to antibiotics. A different gonococcal strain was the cause of the second infection in 53% of the patients. Longer intervals between infections were significantly associated with greater numbers of dissimilar strains as a cause of the second infection. The same strain was usually found in infections occurring within 60 days of each other whereas different strains were more likely to cause infections occurring more than 60 days apart. The isolates causing the second infection were significantly more susceptible to penicillin.

Introduction

Patients with repeated gonococcal infections are responsible for a large number of the reported cases of gonorrhoea in venereal disease clinic patients.[1 2] The frequency of reinfection in these groups of patients has resulted in the belief that immunity to gonococcal infections may be minimal. Although both serological and humoral immune responses to *Neisseria gonorrhoeae* have been found in patients with genital infections,[3-6] there is doubt that these responses are adequate to protect patients from a second infection.[3 4] Studies of patients with repeated episodes of gonorrhoea have not examined the importance of time as a variable in the similarity of the gonococcal strain isolated from the initial and subsequent infection.

In the present study, we examined gonococci isolated from patients with repeated episodes of gonorrhoea which occurred at varying intervals. We wished to identify the frequency with which the strain of the first and second gonococcal isolate differed. Since no definitive serotyping or immunotyping system has been universally accepted for gonococcal taxonomy, we chose auxotyping and antibiotic susceptibility testing as the methods for characterising the gonococcal isolates.

Patients and methods

BACTERIAL ISOLATES

The patients were selected from the Lexington-Fayette County Venereal Disease Clinic, Lexington, Kentucky, USA, between December 1973 and October 1977 and from the Venereal Disease Clinic of the Erasmus University Hospital (Dijkzigt), Rotterdam, the Netherlands, between September 1977 and April 1978. The former clinic serves people of primarily one county in Kentucky and the latter sailors, prostitutes, and other citizens of a large seaport. The patients were those individuals who had more than one gonococcal infection during the study period and whose gonococcal isolates could be saved. The 38 patients selected for study had infections with *N gonorrhoeae* confirmed by cultures at separate clinic visits which took place from one to 185 weeks apart. The cultures giving positive results were from the same anatomical site in 32 of the 38 patients.

All patients were treated with antibiotics after the initial positive culture and 21 of the 38 patients had one or more cultures performed between the two infections. The patients attending the Lexington

Address for reprints: Dr R C Noble, Division of Infectious Diseases, Department of Medicine, University of Kentucky College of Medicine, Lexington, Kentucky 40536, USA

Received for publication 29 June 1979

clinic received one of three drug regimens; aqueous procaine penicillin G 4·8 megaunits i.m. plus probenecid 1 g orally; ampicillin 3·5 g plus probenecid 1 g orally; or tetracycline 1·5 g orally followed by 0·5 g four times daily for four days. The patients at the Rotterdam clinic received amoxycillin 3 g plus probenecid 1 g orally.

The gonococci were cultured initially on modified Thayer-Martin medium.[7] GC medium base (Difco Laboratories, Detroit, Michigan) with 1% IsoVitaleX (Baltimore Biological Laboratory, Cockeysville, Maryland) was used as the laboratory study medium. Gonococci were identified by means of colonial morphology, oxidase reaction, Gram stain, and carbohydrate reactions. The bacteria were stored at −70°C in tryptic soy broth (Difco) containing 15% glycerol. Because of the possibility that more than one gonoccal strain might infect a patient at the same time,[8-10] three separate colonies were studied from the initial and final cultures from patients attending the Rotterdam clinic. Where it was possible, a second isolate from a separate site on the same patient was also examined. This isolate was usually one which had been cultured at the time of the initial visit.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The antibiotic susceptibility of the gonococcal isolates to two-fold dilutions of ampicillin, penicillin, and tetracycline was tested by one of two agar plate methods as previously described.[11 12] In addition, the isolates from the Lexington clinic were tested for susceptibility to spectinomycin.[12]

GONOCOCCAL AUXOTYPING

Gonococcal auxotyping was performed by the method of Catlin[13] and Carifo and Catlin.[14] Gonococci of known auxotype were included in each test.[12]

ANALYSIS OF DATA

Isolates from a single patient were always tested together. Gonococcal isolates were identified as separate strains if they had different auxotypes or if one or more of the antibiotic susceptibility test results differed by more than two dilutions. Analysis of the categorical data was done by the χ^2 method and the antibiotic susceptibility data were analysed by the Wilcoxon matched-pairs signed-ranks test.[15]

Results

The patients consisted of 14 men and 24 women from the two clinics (Rotterdam, four men and 19 women; Lexington 10 men and five women). Thirteen women gave their occupation as prostitute, and these were all from the Rotterdam clinic. All intervening cultures gave negative results for the 21 patients who had cultures performed after antibiotic therapy for their initial infection. Cultures were not performed for the other 17 patients between their first and second infections.

GONOCOCCAL STRAINS

Fifty-three per cent (20/38) of the patients had a different gonococcal strain as the cause of their second infection. These 20 strains differed from the initial strain in the following respects: one had a different auxotype and was not tested for antibiotic susceptibility; eight, different auxotypes and antibiotic susceptibilities; nine, the same auxotype and different antibiotic susceptibilities; and two, different auxotypes and the same antibiotic susceptibilities. Of the 23 patients from the Rotterdam clinic, the same strain was found in the three colonies from each culture. However, two of these 23 patients were infected simultaneously with two strains that were identified by cultures from separate sites on the same patient during the same visit.

There was no statistically significant difference between the sexes in the number of different strains found with the second infection. Different strains were found in 57% (8/14) of the men and 50% (12/24) of the women with their second infection. Different gonococcal strains were found in 46% (6/13) of the prostitutes and 54% (6/11) of the other women, and this difference was also not statistically significant. The relationship of the interval between the two infections and the similarity of the strains is shown in the Table. Longer intervals were significantly associated with greater numbers of dissimilar strains as a cause of the second infection.

TABLE *Relationship of interval between infection and the presence of a different gonococcal strain in patients with recurrent gonorrhoea*

Interval (days)	*% of strains*		*Total No*
	Alike	*Different*	
<30	71·4	28·6	14
30-60	55·5	44·4	9
>60	20·0	80·0	15

$\chi^2_2 = 7\cdot98$; $P<0\cdot02$

ANTIBIOTIC SUSCEPTIBILITIES

The antibiotic susceptibility of the second isolate was compared with that of the first in 37 patients. Differences in susceptibility to one or more antibiotics were present in the paired isolates of 17 patients. There were differences in susceptibility to penicillin in 15 pairs, to ampicillin in 14 pairs, to tetracycline in 11 pairs, and to spectinomycin in seven pairs from these 17 patients. In these pairs in which there was a difference in susceptibility, the second isolate was more susceptible to penicillin in 80% (12/15) ($P<0 \cdot 05$), to ampicillin in 71% (10/14), to tetracycline in 73% (8/11), and to spectinomycin in 71% (5/7). Except for penicillin, these differences in susceptibility were not statistically significant. The distribution of the auxotypes of the first and second group of isolates was also not significantly different.

Discussion

Unlike many other infectious diseases, gonorrhoea frequently recurs in the same patient. Repeated episodes of gonorrhoea may be the result of an inadequate host defence mechanism, or possibly repeated infections may be caused by different strains, each strain producing only type-specific immunity. Since there is disagreement on the antigen or antigens which may be responsible for the latter, we chose non-immunological markers for characterising the paired isolates. Both auxotyping and antibiotic susceptibility test results are reproducible in isolates taken from the same patient.[8 9 12 16 17]

Only a few studies have examined the characteristics of gonococci isolated from patients with repeated infections, and the influence of time on the similarity of the repeat isolates has not been emphasised. We have re-analysed data from four studies in which the interval between infections was mentioned. Tramont *et al*[18] examined isolates from five women and one man with 10 episodes of repeated gonococcal infections. The strains were characterised by an in-vitro bactericidal assay using a set of 20 typing antisera to *Neisseria meningitidis*. Repeated infections occurring within a 60-day period were predominantly with the same strain (4/5) whereas repeated infections occurring more than 60 days apart were predominantly with different strains (4/5). Silver and Darling[19] examined gonococcal isolates from 20 episodes of recurrent infection after periods ranging from 4 to 119 days. The gonococci were characterised only by their susceptibility to penicillin G. In 15, the susceptibility of the second isolate was identical to that of the first; and, in five, there was only a difference of one dilution towards greater resistance. Carifo and Catlin[14] examined gonococci from one man and three women, who had a total of seven repeated infections. The isolates were characterised by auxotyping and susceptibility to penicillin G. Five of the episodes occurred within 30 days. All isolates had the same auxotype and the penicillin susceptibilities were all within one dilution. Two episodes occurred more than 130 days apart; and, in one of the two, the auxotype and penicillin susceptibility differed. In the report of Catlin and Pace,[17] 25 patients had recurrent oropharyngeal gonococcal infections. The isolates were characterised by auxotyping and susceptibility to penicillin. The interval between infections was not stated for all isolates. All except one of the paired isolates had the same auxotype and penicillin susceptibility.

The finding that the second gonococcal isolate tended to be more susceptible to the antibiotics was unexpected. Susceptibility to penicillin of gonococcal isolates has been increasing in the United States[20] as well as in the Netherlands (Stolz, unpublished observations) since 1972, and this phenomenon has been attributed to better treatment schedules since that time.[20] A similar parallel was found in Greenland; the susceptibility of gonococcal strains to penicillin increased significantly between 1964 and 1967 after an intensive treatment and contact-tracing campaign.[21] The appearance of penicillin-susceptible strains has been associated with the increased frequency of isolation of gonococci with an auxotype that was more susceptible to penicillin.[22] However, the distribution of auxotypes of our initial and final isolates was not significantly different.

In the present study, longer intervals between infections were associated with a dissimilarity of the gonococcal strains causing the repeat infections. In infections occurring within a 60-day period, most of the paired isolates were the same. This difference was most striking during the first 30 days. We believe that few if any of these isolates represented treatment failures because all patients were treated with adequate antibiotic regimens,[23 24] and all the patients who had cultures performed after treatment had one or more negative results. Infection with the same strain during the first 60-day period was probably the result of resumption of sexual contact with an untreated partner. Prostitutes were not infected more frequently with a different strain than other women. Although prostitutes undoubtedly had more sexual partners than the other women, infection could also have been acquired from an untreated regular client or friend. Other explanations for this result may be that either there were only a limited number of different gonococcal strains in the community within the study period or the methods used to study the gonococci were not sensitive enough to detect other differences between the isolates.

Thus, recurrent gonococcal infections occurring within a 60-day period are more commonly caused by the same gonococcal strain. After this interval, recurrent infections are more common with a different strain.

We are grateful for the technical assistance of Mrs Betty R Miller, Mej J C de Roo, Mevr G M R Bosscher-Koetsier, and Mevr M P E de Jonge-Suij. We thank Mrs A M Noble for her review and suggestions. We are also grateful to Dr R van Strik, Department of Biostatistics, Erasmus University, Rotterdam, for his help in the statistical analysis. The study was made possible by funds provided by the Erasmus University, Rotterdam, Research Grant AI 11304 from the National Institute of Allergy and Infectious Disease, and a grant from the Alexandrine and Alexander Sinsheimer Fund. We also thank Mr Steven F Clark and the other members of the Lexington-Fayette County Health Department, whose co-operation made this study possible.

References

1. Noble RC, Kirk NM, Slagel WA, Vance BJ, Somes GW. Recidivism among patients with gonococcal infection presenting to a venereal disease clinic. *Sex Transm Dis* 1977; **4:** 39-43.
2. Brooks GF, Darrow WW, Day JA. Repeated gonorrhea: An analysis of importance and risk factors. *J Infect Dis* 1978; **137:** 161-9.
3. Kearns DH, Seibert GB, O'Reilly RO, Lee L, Logan L. Paradox of the immune response to uncomplicated gonococcal urethritis. *N Engl J Med* 1973; **289:** 1170-4.
4. Brooks GF, Ingwer I. Studies on the relationships between serum bactericidal activity and uncomplicated genital infections due to *Neisseria gonorrhoeae*. *J Infect Dis* 1978; **138:** 333-9.
5. Bisno AL, Ofek I, Beachey EH, Curran JW. Human immunity to gonococci: studies of opsonization by sera of prostitutes and nuns. *Trans Assoc Am Physicians* 1974; **87:** 195-204.
6. Kasper DL, Rice PA, McCormack WM. Bactericidal antibody in genital infection due to *Neisseria gonorrhoeae*. *J Infect Dis* 1977; **135:** 243-51.
7. Martin JE, Lester A. Transgrow a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Health Services and Mental Health Administration Health Reports* 1971; **86:** 30-33.
8. Catlin BW, Pace PJ. Auxotypes and penicillin susceptibilities of *Neisseria gonorrhoeae* isolated from patients with gonorrhea involving two or more sites. *Antimicrob Agents Chemother* 1977; **12:** 147-56.
9. Short HB, Ploscowe, VB, Weiss JA, Young FE. Rapid method for auxotyping multiple strains of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1977; **6:** 244-8.
10. Noble RC. Characterisation of *Neisseria gonorrhoeae* from women with simultaneous infections at two sites. *Br J Vener Dis* 1980; **56:** 3-5.
11. Stolz E, Zwart HGF, Michel MF. Activity of eight antimicrobial agents in vitro against *N gonorrhoeae*. *Br J Vener Dis* 1975; **51:** 257-64.
12. Noble RC, Schell SC. Acrylamide gel electrophoresis of proteins of *Neisseria gonorrhoeae* as an epidemiological tool. *Infect Immun* 1978; **19:** 178-86.
13. Catlin BW. Nutritional profiles of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. *J Infect Dis* 1973; **128:** 178-93.
14. Carifo K, Catlin BW. *Neisseria gonorrhoeae* auxotyping: differentiation of clinical isolates based on growth responses on chemically defined media. *Appl Environ Microbiol* 1973; **26:** 233-30.
15. Siegel S. *Nonparametric Statistics for the Behavioral Sciences*. New York: MacGraw Hill, 1956.
16. Reyn A, Bentzon MW. Sensitivity to antibiotics of gonococcal strains isolated by repeated culturing from the same patient. *Acta Derm Venereol (Stockh)* 1963; **43:** 394-8.
17. Catlin BW, Pace, PJ. Nutritional requirements and penicillin susceptibilities of gonococci from pharyngeal and anogenital sites. *Br J Vener Dis* 1977; **53:** 299-303.
18. Tramont EC, Griffiss JM, Rose D, Brooks GF, Artenstein MS. Clinical correlation of strain differentiation of *Neisseria gonorrhoeae*. *J Infect Dis* 1976; **134:** 128-34.
19. Silver PS, Darling WM. Penicillin-insensitive gonococci in the Bolton area. Preponderance in young women and immigrants. *Br J Vener Dis* 1971; **47:** 367-72.
20. World Health Organisation. *Neisseria gonorrhoeae* and gonococcal infections. *WHO Technical Report Series* 616. Geneva: WHO, 1978.
21. Olsen GA. Consumption of antibiotics in Greenland, 1964-70. IV. Changes in the sensitivity of *N gonorrhoeae* to antibiotics. *Br J Vener Dis* 1973; **49:** 33-41.
22. Eisenstein BI, Lee TJ, Sparling PF. Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated infection. *Infect Immun* 1977; **15:** 834-41.
23. Kaufman RE, Johnson RE, Jaffe HW, *et al.* National gonorrhea therapy monitoring study. Treatment results. *N Engl J Med* 1976; **294:** 1-4.
24. Center for Disease Control. Gonorrhea. CDC recommended treatment schedules. *Morbidity and Mortality Weekly Report* 1979; **28:** 13-16.

British Journal of Venereal Diseases, 1980, **56,** 35-36

An unusual case of gonococcal arthritis

K M RENNEY

From the Department of Family Practice, San Bernardino County Medical Center, San Bernardino, California, USA

SUMMARY In cases of gonococcal arthritis the knees, wrists, ankles, hands, and feet are the joints most commonly affected. This case is unusual because of the occurrence of gonococcal arthritis in the sacroiliac joint.

Introduction

Arthritis is a common complication of gonococcal infection. The incidence is about 1% in patients who develop gonorrhoea.[1] The joints most commonly affected are the knees, wrists, ankles, and the metacarpophalangeal and metatarsophalangeal joints.[2] In the present case gonococcal arthritis of the right sacroiliac joint was found, which is an unusual occurrence.

Case report

A 21-year-old black woman was admitted on 26 September 1976 with a seven-day history of migratory joint pains finally resulting in severe pain in the right hip. The patient stated that the joint pain had begun in the right thumb and had progressed to her left ankle, and right hip, knee, and ankle. Each joint had become swollen, reddened, and very tender for limited periods of time. She denied any recent history of upper respiratory illness, kidney infection, or exposure to gonorrhoea or syphilis.

On clinical examination the patient was in moderate distress. Her temperature was 37·4°C, pulse rate 96 beats/min, and blood pressure 140/88 mm Hg. On examination of the joints and extremities there was no increased warmth, redness, or effusion over any previously affected joints. The range of movement of the right hip was limited because of pain and there was point tenderness over the right sacroiliac region. The patient was unable to bear weight on the right leg owing to the severe pain in the right hip with radiation to the sacral region.

White blood count was $7 \cdot 7 \times 10^3/\text{mm}^3$ ($7 \cdot 7 \times 10^9/\text{l}$) with 71% neutrophils, packed cell volume 36·5%, and erythrocyte sedimentation rate 37 mm/h. The ASO titre was reported as 50 Todd units. Additional investigations giving negative results were antinuclear antibodies (ANA), lupus erythematosus cells, rheumatoid factor, Monotest, Venereal Disease Research Laboratory test, DriDot pregnancy test, and sickle-cell screen. Urine analysis showed 5-7 polymorphonuclear leucocytes per high power field and acetone. Electrolyte levels were normal; uric acid was 0·09 mmol/l and salicylate level was 2·64 nmol/l.

Radiographs of the chest, lumbosacral spine, sacroiliac joint, pelvis, and right hip showed no abnormalities. An electrocardiogram was normal.

After specimens for culture for gonococci from the cervix, vagina, and rectum, together with specimens of blood and urine, had been obtained, the patient was rested in bed and treated with acetylsalicylic acid 600 mg every four hours.

An orthopaedic surgeon confirmed the absence of an effusion in the right hip and the presence of point tenderness over the right sacroiliac joint and suggested a Tc-99 bone scan.

Meanwhile, results of the blood cultures were negative; throat and vaginal flora were normal with no growth of *Neisseria gonorrhoeae.*

On day 4 after admission the bone scan showed increased isotope uptake in the right sacroiliac area. After consultation with the rheumatologists treatment with acetylcalicylic acid was continued and indomethacin 25 mg three times daily added to the regimen.

The patient's condition remained unchanged and on day 10 a Craig needle-biopsy of the right sacroiliac joint was performed. The material from the biopsy was submitted for routine aerobic and anaerobic culture, culture on Thayer-Martin medium for gonococci, culture for acid-fast bacilli, and histological studies. Suspicious colonies on Thayer-Martin medium were evaluated by Gram staining and biochemical tests.

Address for reprints: Dr K M Renney, 7651 Highland Woods CT/2b, Lorton, Virginia 22079, USA

Received for publication 17 January 1979

On day 13, the patient continued to have pain; the treatment was changed from indomethacin to phenylbutazone 100 mg three times daily. Further laboratory results showed a C3 complement level of 257 mg/dl, a negative response for HLA-B27, and normal haemoglobin electrophoresis. The sacroiliac biopsy showed chronic, non-specific synovitis.

After four days' treatment with phenylbutazone, the patient's condition had improved slightly and she was able to bear weight on crutches despite pain in the right hip and sacroiliac region. On 13 October 1976 the patient was discharged; she was to continue taking phenylbutazone and acetylsalicylic acid.

On 15 October 1976, when she returned for follow-up examination, the biopsy culture showed growth of *N gonorrhoeae*. The patient was readmitted and treatment with aqueous penicillin 500 000 units i.v. every four hours was started and continued for 10 days. The patient's condition promptly improved. On day 10 after readmission, she was discharged free of symptoms and had a full range of movement in the right hip.

Comment

The unusual feature of this case is the involvement of the sacroiliac joint. Only eight reports relating to gonococcal arthritis since 1919 mention sacroiliac joint disease, confirming its rarity. Michel[3] reported five cases of sacroiliac joint disease in his series of 100 patients and Wehrbein[4] found 15 in a series of 610 cases. Later in the 1930s, Myers and Gwynn[5] reported nine in their series of 85 cases, Keefer and Spink[6] found eight cases in their study of 140, and Burbacher and Weiland[7] mentioned sacroiliac joint disease in their series of 58 cases. In 1949, Culp[8] reported one case in his study of 200 cases, in 1942 Harkness[9] reported 17 out of 336 patients, and in 1974 Seifert[10] reported severe sacroiliac joint pain in one of his 16 cases.

During the last decade, many of the classical concepts of gonococcal arthritis have changed, mostly because of a clearer understanding of the clinical features of this disease and a greater accuracy in diagnosis.[11] Thus many of the earlier case reports may have contained a potential source of confusion with what we today recognise as Reiter's syndrome. Similarly, patients with Reiter's syndrome may have had *N gonorrhoeae* coincidently identified in their genitourinary tract. This may account for the occasional case of gonococcal arthritis that failed to respond to penicillin therapy.[2 12] Today we are able to separate Reiter's syndrome as a distinct clinical entity. Thus, even if Reiter's syndrome may have been a cause of confusion in these earlier reports, sacroiliac joint involvement was still an unusual occurrence.

In addition to joint involvement, this case indicates the importance of taking samples for culture from several sites to obtain a bacteriological diagnosis in a symptomatic patient. Although some authors suggest that treatment of gonococcal arthritis should be based on the clinical picture alone, it is also essential to make a bacteriological diagnosis, either by culture or by more invasive procedures such as joint aspiration or biopsy. This bacteriological information differentiates gonococcal infection from other types of articular disease.

References

1. Keiser H, Ruben FL, Wolinsky E, *et al.* Clinical terms of gonococcal arthritis. *New Engl J Med* 1968; **279:** 234-40.
2. Wright V. Arthritis associated with venereal disease. A comparative study of gonococcal arthritis and Reiter's syndrome. *Ann Rheum Dis* 1963; **22:** 77-90.
3. Michel LL. Infectious arthritis of urogenital focal origin. *NY State J Med* 1919; **109:** 1124.
4. Wehrbein H. Gonococcus arthritis: a study of 610 cases. *Surg Gynecol Obstet* 1929; **49:** 105-13.
5. Myers WK, Gwynn HB. Clinical features of gonococcal arthritis; observations in 85 cases. *Med Ann DC* 1935; **4:** 194-97.
6. Keefer CS, Spink WW. Gonococcic arthritis: pathogenesis, mechanism of recovery and treatment. *JAMA* 1937; **109:** 1448-53.
7. Burbacher CR, Weiland AH. Gonorrheal arthritis. *J Fla Med Assoc* 1938; **24:** 433-6.
8. Culp OS. Treatment of gonococcal arthritis: analysis of 200 cases. *J Urol* 1940; **43:** 737-65.
9. Harkness AH. Gonococcal arthritis. *Med Press* 1942; **207:** 60-4.
10. Seifert MH, Warin AP, Miller A. Articular and cutaneous manifestations of gonorrhoea. Review of sixteen cases. *Ann Rheum Dis* 1974; **33:** 140-6.
11. Kushner I. Gonococcal arthritis. *Med Times* 1970; **98:** 111-16.
12. Holmes KK, Counts GW, Geaty HN. Disseminated gonococcal infection. *Ann Intern Med* 1971; **74:** 979-93.

British Journal of Venereal Diseases, 1980, **56,** 37-45

Chlamydial infection of the cervix in contacts of men with nongonococcal urethritis

I ANNE TAIT,* ELISABETH REES,* D HOBSON,† RUTH E BYNG,†
AND M C K TWEEDIE‡

*From the Departments of *Venereology, †Medical Microbiology, and ‡Biostatistics, University of Liverpool, Royal Liverpool Hospital, Liverpool*

SUMMARY An investigation of chlamydial infection in sexual contacts of patients with nongonococcal urethritis (NGU) was carried out to determine the clinical signs of infection in the cervix, and their response to chemotherapy, and the incidence of cervical infection in the presence of ectopy and oral contraception.

In 202 consecutive female contacts of NGU the isolation rate of *Chlamydia trachomatis* was 35%. Hypertrophic ectopy and endocervical mucopus were present in 19% and 37% of chlamydia-positive patients respectively and, in all but one, resolved after treatment. Only 14% of those followed up after treatment developed yeast infections. The chlamydial isolation rate was significantly higher in patients with hypertrophic ectopy and endocervical mucopus. Cervical ectopy and oral contraceptives acted additively, each producing a significant effect on the chlamydial isolation rate in the presence of the other but not when present alone.

Introduction

The clinical diagnosis of nongonococcal urethritis (NGU) in men is often arbitrary and commonly based solely on the finding of 10 polymorphonuclear leucocytes per high power field (× 100 objective) in a urethral smear with no evidence of *Neisseria gonorrhoeae.* However, strong evidence, recently reviewed by Schachter,[1] has now accumulated for a causative role for *Chlamydia trachomatis* in up to 50% of men with NGU and for the sexual transference of this agent.

In women, the commonest site of chlamydial infection (as in gonorrhoea) is the cervix,[2 3] the site of greatest coital contact, but *C trachomatis* may also be isolated—although less frequently—from the rectum,[4] the urethra,[5] and Bartholin's ducts.[6]

Criteria similar to those used in the diagnosis of NGU in men cannot be applied to cervicitis because the endocervical secretion contains polymorphonuclear leucocytes which vary in number according to hormonal factors and coital history. Until the clinical signs of infection and related symptoms were investigated in conjunction with rapid tissue culture procedures for chlamydial isolation (based on methods of Gordon and Quan[7]) the diagnosis of nongonococcal cervicitis in women was unsatisfactory owing to the absence of a known aetiological agent.

The difficulties associated with interpreting clinical signs in the cervix have previously been discussed in detail.[8] The same method of categorising the state of the ectocervix is used here; it is based upon the concept that ectopy (erosion) is not a pathological condition but simply ectopic columnar epithelium which extends from the endocervix to the ectocervix.[9 10] The redness which is associated with such ectopy—and sometimes thought to represent inflammation—results from the sub-mucosal capillary bed being viewed through a single layer of columnar epithelium instead of many layers of squamous epithelium. Irregularity of outline with peninsulae and islands of squamous epithelium together with Nabothian follicles (ducts of mucus glands blocked by metaplastic squamous epithelium) indicates metaplasia which is, in most cases, physiological but may be dysplastic.[11] When an area of ectopy is oedematous and congested and bleeds, we have called it "hypertrophic". This appearance was first described as "cobblestone" by Dunlop,[12] who also reported the presence of "follicles" on colposcopical examination of the cervix. Together with mucoid or

Address for reprints: Dr I A Tait, Department of Venereology, Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XP

Received for publication 25 June 1979

mucopurulent endocervical discharge these two signs, singly or combined, indicate endocervicitis.

At present there is no consistent management of sexual contacts of men with NGU. Some physicians refer all contacts for investigation and give treatment to all on epidemiological grounds, irrespective of clinical or microbiological findings. Others are selective in both their referrals and their treatment. Many physicians have been deterred from giving epidemiological treatment—in the absence of symptoms—as candidosis might ensue. However, women with gonorrhoea are commonly asymptomatic and without clinical signs. They are treated when *N gonorrhoeae* is isolated because it has been established that failure to treat may lead to serious consequences for the patient herself and for the community. It is therefore important that the pathogenic role of *C trachomatis* in women should be defined; (a) to establish the need for the treatment of known infections; and (b) to provide information for physicians who may have to manage their patients without isolation facilities.

This paper gives the results of an investigation of chlamydial infection in 202 women who were sexual contacts of men with NGU with particular regard to: (a) the incidence of clinical signs and their response to treatment in patients from whom *C trachomatis* was isolated; (b) the presence of concurrent infection; and (c) the incidence of chlamydial infection in patients with cervical ectopy and in those taking contraceptive steroids and the possible interrelationship of these two factors on the isolation rate of this agent.

Patients and methods

CLINICAL INVESTIGATIONS

The group of women examined consisted of 202 contacts of men with NGU who attended Liverpool Royal Infirmary consecutively on specified days. All the male contacts had been diagnosed in sexually transmitted diseases (STD) clinics where facilities for isolation of *C trachomatis* were not available. Women who had received antibiotics in the four preceding weeks were excluded. Known cases or contacts of gonorrhoea were also excluded as that infection can also cause endocervicitis.

In this group of women, 169 presented with contact slips; the remainder attended of their own accord, or were referred by another doctor, and gave a history that their sexual contact was attending a male STD clinic. In these cases, the diagnosis was always obtained from the clinic named.

Swabs for detection of *N gonorrhoeae* were taken from the urethra and endocervix on at least two separate occasions and used to inoculate a selective medium containing antibiotics (London Analytical and Bacteriological Media Ltd, London) and make a smear for Gram staining. All patients were investigated for syphilis by conventional methods and for trichomoniasis and yeast infection by inoculation of Feinberg-Whittington medium. (Yeast infection was only recorded if cultures gave positive results even when clinical signs and symptoms suggesting the condition were present.) Swabs for culture for *C trachomatis* were taken from the endocervical canal and, in addition, rubbed on any ectopic columnar epithelium as previously described.[6] Swabs were not taken from the urethra.

LABORATORY TESTS

Swabs for culture for *C trachomatis* were immediately placed in 2-3 ml of transport medium[13] and stored directly at 4°C until inoculated into tissue culture. This was usually done two to four hours after the swab had been taken but occasionally after storage for 24 hours.

Swab contents were thoroughly dispersed into transport medium and 0·4 ml were inoculated on to each of two 16-mm coverslip cultures of replicating McCoy cells (that is, cells not pre-treated by *x*-irradiation or any anti-replicative chemical agents). Centrifugation-assisted absorption of the inoculum on to the McCoy cells as well as incubation conditions and method of examination of inoculated cultures after 48-72 hours' incubation have all been described previously.[14]

In all cases, the total number of intracellular inclusions of *C trachomatis* recovered from each clinical specimen on the McCoy coverslip culture was counted. The inclusions detected represent a single cycle of development; their number therefore reflects the number of infective particles in the original cervical swab and is a direct measure of the number of particles being shed from the lesion. The purpose of this quantitative procedure was (a) to check the sensitivity and reproducibility of the laboratory method and (b) to have additional data against which the severity, response to treatment, and possibility of relapse might be assessed.

In all cases, the results represent the primary inoculation of specimens in tissue culture. No second passage from tissue culture to further tissue culture was performed to avoid any risk of false-positive results by cross-contamination.

CLINICAL SIGNS

The clinical signs were recorded on the request form and case sheet at the time of sampling.

The state of the ectocervix has been recorded in three categories: no ectopy, simple ectopy, and hypertrophic ectopy (Figs 1-3). The contents of the

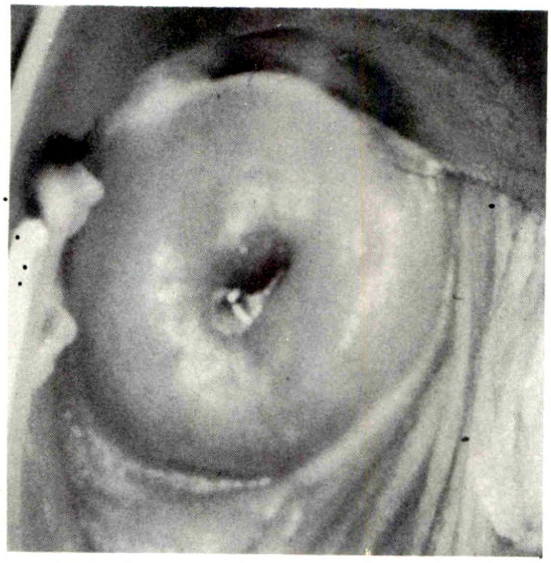


FIG 1 *Cervix showing no ectopy.*

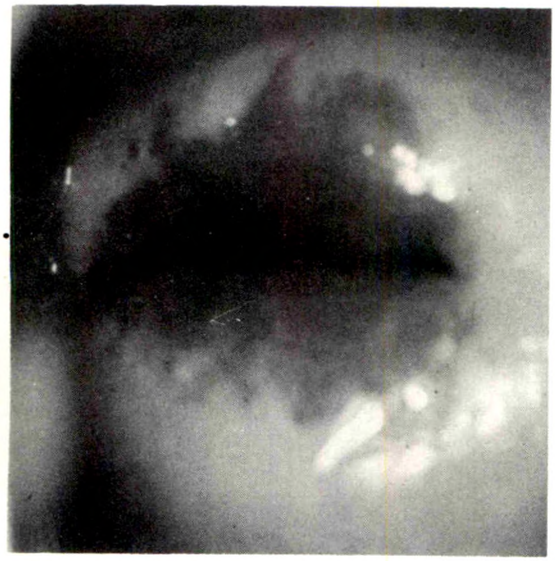


FIG 2 *Cervix showing simple ectopy.*

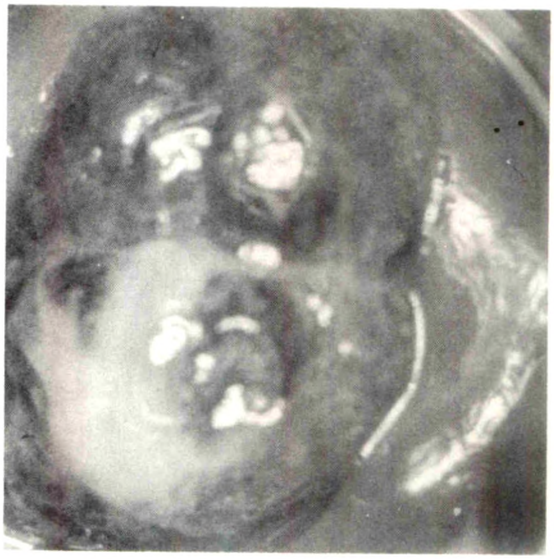


FIG 3 *Cervix showing hypertrophic ectopy with endocervical mucopus. (C trachomatis isolated.)*

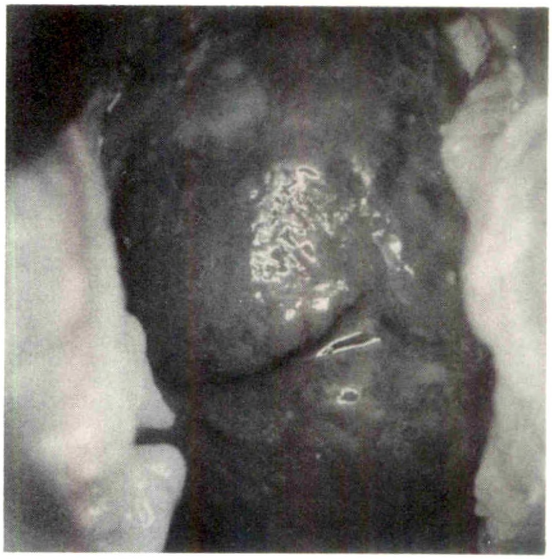


FIG 4 *Cervix showing clear mucus with simple ectopy.*

endocervix have been recorded in three categories; clear mucus, cloudy mucus, and mucoid or mucopurulent discharge (Figs 4 and 5). In examining the cervical contents, care was taken to remove discharge extruding from the cervix before the contents of the canal were assessed and sampled. Cervical secretion spreading from the os into the vagina becomes contaminated by vaginal flora, thus appearing mucopurulent in some cases, whereas behind this clear mucus may be seen in the canal.

TREATMENT

Routine treatment of chlamydia-positive women was with oxytetracycline hydrochloride tablets 250 mg six-hourly for 21 days. Pregnant women were not given oxytetracycline but received erythromycin BP 250 mg six-hourly. Treatment was given for 21 days as this is the longest course advised for men with NGU and we did not want possible inadequate treatment to be a cause of persisting clinical signs or chlamydial reisolation or both.

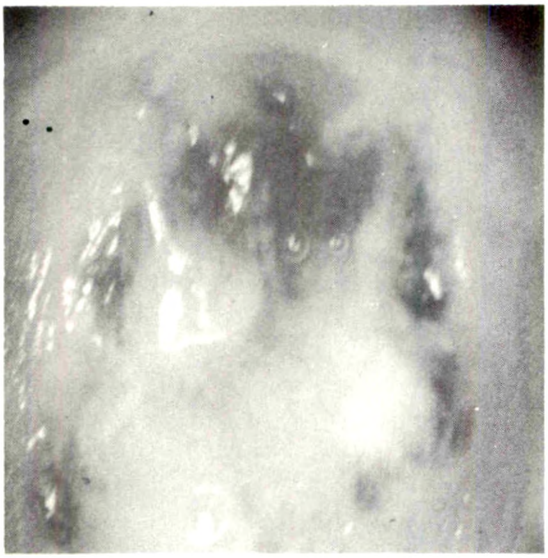


FIG 5 *Cervix showing endocervical mucopus with simple ectopy.*

Patient compliance can be a problem with such prolonged treatment. To assess this, blood samples were taken for serum tetracycline estimation at 14 or 21 days in 26 women. Patients were not told in advance that the test was to be carried out; hence the time of venepuncture varied from two to five hours after the last dose. The range of tetracycline concentrations was 0·53-2·01μg per ml (mean = 1·21). This suggests that patient compliance was good and that the serum concentration was satisfactory (minimum inhibitory concentration of oxytetracycline for *C trachomatis* = 0·2μg/ml[15]).

FOLLOW-UP

All chlamydia-positive patients were asked to attend for review to check whether or not candidosis had developed and to assess the response to treatment on day 14 (of the course of treatment), on day 28 (that is, seven days after completion of treatment), twice more at three-weekly intervals, and subsequently at six weeks, a total of three months' post-treatment observation. Several patients agreed to co-operate by continuing to attend at three-monthly intervals and others returned with fresh symptoms or requesting reinvestigation within that time.

Specimens were taken for culture for *C trachomatis, N gonorrhoeae, Trichomonas vaginalis,* and *Candida* species on each post-treatment visit.

STATISTICAL METHODS

Statistical comparisons were made using the χ^2 test with Yates's correction.

Results

C trachomatis was isolated from 70 (35%) of the 202 women.

AGE

The age range for all 202 women was 16-46 years (mean 25 years); in the chlamydia-positive group it was 16-37 years (mean 23 years).

MARITAL STATE

One hundred and ten women were single (chlamydia-positive 36%) and 68 were married (chlamydia-positive 28%), 23 were divorced or separated (chlamydia-positive 39%), and the status of one was unrecorded.

HORMONAL FACTORS

Pregnancy

Five patients were pregnant; two (one chlamydia-positive) were in the first trimester, two (both chlamydia-negative) in the second, and one (chlamydia-negative) in the third.

Menstrual cycle

No significant difference was found in the chlamydial isolation rates in successive weeks of the menstrual cycle.

In those 184 women with a regular cycle of 28 days (± 2), the month was divided into four weeks, week 1 starting from the first day of menstruation. The week in which the swab for *C trachomatis* was taken was recorded in each case. The isolation rates are shown in Table I. In 18 (21% chlamydia-positive) the cycle was irregular.

TABLE I *Isolation of C trachomatis in relation to the week of the menstrual cycle*

	Menstrual cycle (week)				
Isolation	*1*	*2*	*3*	*4*	*Total*
No of patients	45	66	42	31	184
Chlamydia-positive (%)	17 (38)	23 (35)	15 (36)	9 (29)	64 (35)

Oral contraception

At the time of examination, 99 women (48·7% chlamydia-positive) were taking oral contraceptives. The methods of contraception for all 202 patients and the distribution of these methods in relation to chlamydial isolation is shown in Table II.

The chlamydial isolation rate was significantly higher in women taking oral contraceptives than in (a) those not using oral contraception ($\chi^2_1 = 10 \cdot 44$, $P = 0 \cdot 0012$); (b) those using barrier methods ($\chi^2_1 = 8 \cdot 51$, $P = 0 \cdot 0035$); and (c) those using other methods, excluding barrier methods ($\chi^2_1 = 5 \cdot 91$, $P = 0 \cdot 015$).

TABLE II *Isolation of C trachomatis in relation to method of contraception*

Method of contraception	*No of patients*	*Chlamydia-positive*	
		No	*%*
None	52	15	29
Oral contraception	99	46	49
Stopped oral contraception in past month	7	3	43
Barrier	18	1	5·6
Tubal ligation and IUCD	25	5	20
Not known	1		
Total	202	70	35

IUCD = intrauterine contraceptive device

Despite the difference in percentage isolation between those using barrier methods and those using no contraception, the difference in proportions is not significant at 5% ($\chi^2_1 = 2·90$, $P = 0·089$) but the lack of significance may be due only to the small numbers in the group using barrier methods.

SYMPTOMS

The incidence of symptoms and their distribution in relation to chlamydial isolation is shown in Table III. No significant difference was found in the isolation rates in relation to any particular symptom.

TABLE III *Distribution of symptoms, appearance of the ectocervix, contents of the endocervix, and incidence of concurrent infections in relation to isolation of C trachomatis*

	No of patients	*No chlamydia-positive*	*% of positive*
Symptoms*			
None	103	34	49
Discharge	61	22	31
Irritation and soreness	31	17	24
Urinary	24	11	16
Abdominal pain	17	5	7
Postcoital bleeding	3	1	1·4
Dyspareunia	3	2	3
Appearance of ectocervix			
No ectopy	95	21	30
Simple ectopy	87	36	51
Hypertrophic ectopy	17	13	19
Not known	3	0	0
Total	202	70	100
Endocervical contents			
Clear	101	27	39
Cloudy	56	14	20
Mucopus and mucoid	35	26	37
Blood	10	3	4
Total	202	70	100
Concurrent infections			
None	110	40	57
Candida spp	61	16	23
Trichomonas vaginalis	15	10	14
Warts (cervical and vulval)	10	4	6
Other	0	0	0
Total		70	100

*Patients may have had more than one symptom

CLINICAL SIGNS

Ectocervix

The appearance of the ectocervix in relation to the chlamydial isolation rate is shown in Table III. The isolation rate was significantly higher in those with (a) ectopy (simple and hypertrophic), 47% ($\chi^2_1 = 12·5$, $P = 0·0004$); (b) simple ectopy, 41% ($\chi^2_1 = 6·97$, $P = 0·0083$); and (c) hypertrophic ectopy, 76% ($\chi^2_1 = 17·67$, $P = 0·000026$) than in those with no ectopy, 22%.

Endocervical contents

The distribution of contents in relation to the chlamydial isolation rate is shown in Table III. The isolation rate was significantly higher in those with mucopus (74%) than in those with clear and cloudy mucus (26%) ($\chi^2_1 = 27·14$, $P = 0·0000045$).

Salpingitis

Seventeen patients complained of abdominal pain or discomfort (five were chlamydia-positive). Only four of these had clinical signs of salpingitis (two were chlamydia-positive.)

EFFECTS OF ORAL CONTRACEPTION/CERVICAL ECTOPY ON CHLAMYDIAL ISOLATION

To assess the possible interrelationship between oral contraception and cervical ectopy, both of which individually were found to be highly significant in the isolation of *C trachomatis*, the four possible categories were compared and analysed statistically (for this purpose simple and hypertrophic ectopy were combined) (Table IV):

(1) The isolation rates in patients with (57·1%) and without (30·2%) oral contraception in the presence of cervical ectopy ($\chi^2_1 = 6·06$, $P = 0·014$);

(2) The isolation rates in patients with (57·1%) and without (27·3%) ectopy in those taking oral contraception ($\chi^2_1 = 6·3$, $P = 0·012$);

(3) The isolation rates in women with (27·3%) and without (16·4%) oral contraception in the absence of ectopy ($\chi^2_1 = 0·97$, $P = 0·32$);

(4) The isolation rates in patients with (30·2%) and without (16·4%) ectopy in the absence of oral contraception ($\chi^2_1 = 2·06$, $P = 0·15$).

Thus neither oral contraception in the absence of ectopy, nor ectopy in the absence of oral contraception, is associated with a significant increase in the isolation rate. On the other hand, a significant increase is associated both with oral contraception when ectopy is present and with ectopy when oral contraception is being used. Oral contraception and ectopy may be said to act additively, neither being associated with a significant increase in the isolation rate when acting alone but jointly being associated with a significant increase when acting simultaneously.

The isolation rates in the above categories (Table IV) differ to a very highly significant extent ($\chi^2_3 = 22 \cdot 95$, $P = 0 \cdot 000041$).

TABLE IV *C trachomatis isolation in relation to ectopy and oral contraception*

Comparison	*No of patients*	*Chlamydia-positive*	
		No	*%*
Ectopy	99	45	45·5
With OC	56	32	57·1
Without OC	43	13	30·2
No ectopy	94	19	20·2
With OC	33	9	27·3
Without OC	61	10	16·4
Total			
With OC	89	41	46·1
Without OC	104	23	22·1

OC = oral contraception

CONCURRENT INFECTIONS

The presence of concurrent infection in relation to chlamydial isolation is shown in Table III. Only normal flora was found in only 110 patients (35% chlamydia-positive). A significant increase in the isolation rate was found only in the presence of *T vaginalis* ($\chi^2_1 = 5 \cdot 89$, $P = 0 \cdot 15$). *N gonorrhoeae* was present in seven patients, of whom three were chlamydia-positive at the time of their first visit.

WHOLE-COVERSLIP COUNT (WCC)

The range for the 70 chlamydia-positive patients was 1-100 000 inclusions per whole coverslip. The possibility that the degree of infection (as represented by the WCC) may vary according to conditions such as the presence or absence of oral contraception and clinical signs in the cervix was considered. The mean counts of the WCC in these conditions are given in Table V.

TABLE V *Mean whole-coverslip counts in certain conditions*

Condition	*Mean count*
Oral contraception	
Present	6185
Absent	2071
Hypertrophic ectopy	
Present	4895
Absent	4906
Endocervical mucopus	
Present	10 727
Absent	1745

FOLLOW UP

Thirty-eight women were followed up for 2-14 months. *C trachomatis* was reisolated from five patients (Table VI) and there was a strong possibility of reinfection in four of the five cases.*

TABLE VI *Post-treatment follow up*

	No of patients	*Chlamydial reisolation*
Duration of treatment (months)		
<2	8	2*
2-3	4	1
4-6	15	1
7-8	7	
9-10	1	
11-12	1	1
>12	2	1
No of visits		
1	4	1*
2	11	3*
3	6	
4	8	1
5	4	
6	3	1
7	1	
8	1	

*Two reisolates from one patient

Clinical signs

Ectocervix. Of 12 patients with hypertrophic ectopy, 11 reverted to simple ectopy and metaplasia was complete in one.

Contents of the os. Of those patients followed up after treatment, of 20 endocervices previously containing mucopus, eight became clear, 11 became cloudy, and one contained mucoid material.

Yeast infection

Of 70 chlamydia-positive patients, 16 had positive cultures for *Candida* species when the chlamydial infection was diagnosed; twenty-five patients who had negative results for yeasts initially did not attend for follow-up investigations. Of the remaining 29 chlamydia-positive women with negative results for yeasts, who were treated either with tetracycline hydrochloride or erythromycin (two), four (14%) (all taking tetracycline) developed yeast infection.

Discussion

C trachomatis was isolated from 35% of the 202 female sexual contacts of unselected men with NGU. Isolation rates of 22-42%[3 16-18] have previously been reported in different parts of the world. Differences, however, occur in the reporting of clinical signs in the cervix associated with chlamydial infection. Signs of cervicitis have been recorded in 62-89%[17 19 20] but were also present in 58% of uninfected women who were contacts of NGU[17] and in 56% of control women attending a family planning clinic.[20] However, cervical ectopy (erosion, cervical discontinuity) was included as an abnormal clinical sign in all these reports and may account for these differences.

---

*Details of personal case histories may be obtained from Dr I A Tait.

The site of the squamocolumnar junction is laid down at the time of organogenesis and ectopy is found in infants, children, and virgins.[9] At certain times, particularly at puberty and first pregnancy, the ectopic columnar epithelium is replaced by squamous epithelium (squamous metaplasia, Fig 6) with the result that ectopy is less common in the older woman.[9] In a study planned to determine the prevalence and significance of cervical ectopy in women attending family planning clinics in Edinburgh, ectopy was found in 37% of 1400 women and was significantly associated with younger age groups, greater parity, and oral contraception.[21] There was no significant association with the presence of aerobic or anaerobic bacteria, *T vaginalis,* or yeasts.

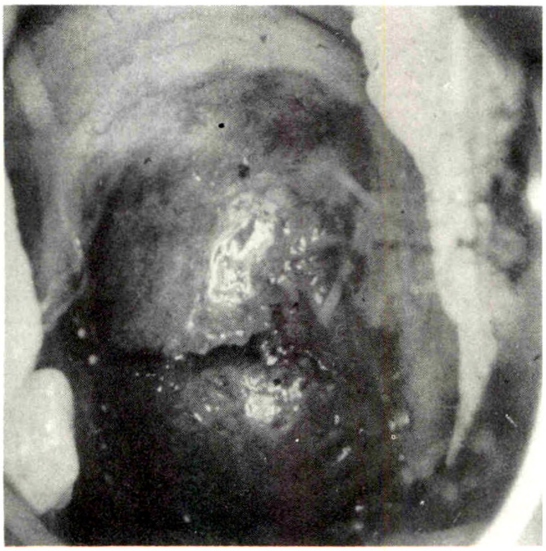


FIG 6 *Cervix showing metaplasia and simple ectopy with clear mucus.*

If signs of cervicitis are narrowly defined on macroscopic examination as endocervical pus within the canal and oedema of the areas covered by columnar epithelium (hypertrophic ectopy or cobblestone appearance as described by Dunlop)[12] there is a highly significant association between cervicitis and the isolation of *C trachomatis.* In this series of consecutive cases, mucopus was found in 37% of chlamydia-positive cases compared with 7% of chlamydia-negative cases (P = 0·0000045), and oedema (hypertrophic ectopy) in 19% of chlamydia-positive compared with only 3% of chlamydia-negative cases (P = 0·000026). The incidence of endocervical mucopus is similar to that found in women with chlamydia-negative gonorrhoea examined in this unit. In each infection, macrosopic evidence of the endocervical infection is absent in 50-60%. After treatment of the chlamydial infection, clinical signs of oedema and mucopus disappeared in all but one patient.

Symptoms associated with endocervicitis are difficult to interpret because the area of susceptible columnar epithelium, and therefore the consequent amount of mucopus which may be produced, is relatively small. Excessive mucus production by the cervical columnar epithelium due to the progestational effect of contraceptive steroids, or due to a large area of ectopy, may result in a discharge which is not associated with infection of any kind. In addition, even a mild vaginitis due to common vaginal pathogens such as *T vaginalis* or yeasts may result in a noticeable discharge owing to the large area of squamous epithelium of the vagina affected.

Consequently, symptoms may bear little relationship to cervical pathology in a large number of women. In this series, no significant relationship was found between the presence of chlamydial infection in the cervix and any of the symptoms. Similar findings have been reported by others.[20 22 23] Indeed Burns *et al*[16] reported a significant absence of symptoms in the presence of chlamydial infection. However, Paavonen *et al*[19] found chlamydia-positive gynaecological patients had significantly more ocular and urethral symptoms than chlamydia-negative patients.

Thus, *C trachomatis* infection appears to be associated with certain well-defined signs of cervicitis in some patients and probably these signs are caused by that infection. On the other hand, with ectopy there is also a definite relation to chlamydial infection but, for reasons already discussed, simple ectopy cannot be considered to be part of the sign-complex of cervicitis. It seems possible that ectopy may be one of many possible interrelated factors which allow infection to be more easily acquired. For example, the virulence of particular serotypes of chlamydia, the immune status of the patient, and anatomical and histological variations may all influence the ease with which infection of the tissues is established. Harkness[24] found a higher prevalence of hypospadias (a condition in which columnar epithelium extends to the external urethral surface since the fossa navicularis is absent) in patients with NGU and gonorrhoea than in the normal population. Cervical ectopy may have a similar effect in chlamydial infection and may be more important than in gonorrhoea because of the intracellular developmental cycle of chlamydia.

Our findings indicate that only in patients in whom the two factors, contraceptive steroids and ectopy, are present is there a significant increase in the isolation rate of *C trachomatis* ($P = 0 \cdot 00004$). Neither factor in the absence of the other is significant. Apart from an implied absence of any barrier to infection of the cervix, oral contraceptive steroids may facilitate cervical infection by their progestational effect, which is similar to that produced in the first trimester of pregnancy. They produce an excessive outflow of alkaline mucus from the endocervical glands and increase the bulk of the cervix, producing eversion with further ectopy. It is noteworthy that steroids added to McCoy tissues cultures infected with *C trachomatis* increase the size and number of inclusions developing.[25]

The interdependence of several varying factors may explain some of the disparity about the effect of oral contraceptives on the chlamydial isolation rate previously reported.[16 17 20 23]

Like Oriel *et al*,[23] but unlike Hilton *et al*,[20] we found no variation in the isolation rate according to the week of the menstrual cycle.

The increased chlamydial isolation rate in the presence of *T vaginalis* may reflect the frequency with which multiple sexually transmissible agents are isolated in promiscuous patients.[26] Others have found no relation between the chlamydial isolation rate and any other concurrent nongonococcal infection.[23]

The mean whole-coverslip counts were raised in patients taking oral contraceptive steroids and in the presence of endocervical mucopus but not in the presence of hypertrophic ectopy. It is possible that oral contraceptive steroids facilitate chlamydial cervical infection, and the presence or absence of endocervical mucopus indicates the severity of the infection, whereas hypertrophic ectopy merely indicates a general host-response to infection in some patients. The possible influence of these clinical factors on the degree of chlamydial replication in infected women will be discussed separately.

In addition to causing acute cervicitis *C trachomatis* has been isolated from the fallopian tubes in cases of pelvic inflammatory disease confirmed at laparoscopy and associated with a fourfold rise in chlamydial antibody titre.[27] Clearly, it is potentially a serious pathogen in women.

This work was supported by a project grant from the Medical Research Council. We would like to thank Dr C D Alergant, Dr E Annels, Dr O P Arya and Dr F Lancely for allowing us access to their male patients' case sheets. We are indebted to Professor Percival and Dr R E Rewell for microbiological investigations, to Dr G C Turner for serological tests for syphilis, to Dr T N Calvey of the Department of Clinical Pharmacology for serum tetracycline estimations, and to Mr C R West for help with statistical analysis.

References

1. Schachter J. Chlamydial infections. *N Engl J Med* 1978; **298:** 428-34.
2. Dunlop EMC, Goldmeier D, Darougar S, Jones BR. *Chlamydia* infection of the genital tract in the mothers and fathers of babies suffering from ophthalmia neonatorum due to TRIC agent. In: Catterall RD, Nicol CS (eds). *Sexually Transmitted Diseases*. London: Academic Press, 1976: 83-8.
3. Terho P. *Chlamydia trachomatis* in non-specific urethritis. *Br J Vener Dis* 1978; **54:** 251-6.
4. Dunlop EMC, Hare MJ, Darougar S, Jones BR, Rice NSC. Detection of *Chlamydia (Bedsonia)* in certain infections of man. II Clinical study of genital tract, eye, rectum and other sites of recovery of *Chlamydia*. *J Infect Dis* 1969; **120:** 463.
5. Dunlop EMC, Darougar S, Hare MJ, Treharne JD, Dwyer RStC. Isolation of *Chlamydia* from the urethra of a woman. *Br Med J* 1972; **7:** 386.
6. Davies JA, Rees E, Hobson D, Karayiannis P. Isolation of *Chlamydia trachomatis* from Bartholin's ducts. *Br J Vener Dis* 1978; **54:** 409-13.
7. Gordon FB, Quan AL. Isolation of the trachoma agent in cell culture. *Proc Soc Exp Biol Med* 1965; **118:** 254-9.
8. Rees E, Tait IA, Hobson D, Johnson FWA. *Chlamydia* in relation to cervical infection and pelvic inflammatory disease. In: Hobson D, Holmes KK, (eds). *Non-gonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977: 67-76.
9. Coppleson M, Reid B. *Preclinical Carcinoma of the Cervix Uteri*. 1st ed. Oxford: Pergamon Press, 1967.
10. Singer A, Jordan JA. The anatomy of the cervix. In: Jordan JA, Singer A, eds. *The Cervix*. London: WB Sanders 1976: 13-36.
11. Singer A. The cervical epithelium during puberty and adolescence. In: Jordan JA, Singer A, (eds). *The Cervix*. London: WB Sanders 1976: 87-104.
12. Dunlop EMC, Al-Hussaini MK, Freedman A, *et al*. Infection by TRIC agent and other members of the Bedsonia group; Genital infection and disease of the eye. *Trans Ophthalmol Soc UK* 1966; **86:** 321-34.
13. Richmond SJ. The isolation of *Chlamydia* sub group A *(Chlamydia trachomatis)* in irradiated McCoy cells. *Med Lab Technol* 1974; **31:** 7-9.
14. Johnson FWA, Hobson D. Factors affecting the sensitivity of replicating McCoy cells in the isolation and growth of *Chlamydia A*. *J Hyg (Lond)* 1976; **76:** 441-51.
15. Treharne JD, Day J, Yeo CK, Jones BR, Squires S. Susceptibility of *Chlamydia* to chemotherapeutic agents. In: Hobson D, Holmes KK, (eds). *Non-gonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977: 214-22.
16. Burns DCM, Darougar S, Thin RN, Lothian L, Nicol CS. Isolation of *Chlamydia* from women attending a clinic for sexually transmitted disease. *Br J Vener Dis* 1975; **51:** 314-8.
17. Oriel JD, Reeve P, Miller A, Nicol CS. Chlamydial infections of the cervix. *Br J Vener Dis* 1974; **50:** 11-16.
18. Holmes, KK, Handsfield HH, Wang S-P, *et al*. Etiology of non-gonococcal urethritis. *N Engl J Med* 1975; **292:** 1199-205.
19. Paavonen J, Saikku P, Vesterinen E, Meyer B, Vartainen E, Saksela E. Genital chlamydial infections in patients attending a gynaecological outpatient clinic. *Br J Vener Dis* 1978; **54:** 257-61.

20. Hilton AL, Richmond SJ, Milne JD, Hindley F, Clarke SKR. *Chlamydia A* in the female genital tract. *Br J Vener Dis* 1974; **50:** 1-10.
21. Goldacre MJ, Loudon N, Watt B, *et al.* Epidemiology and clinical significance of cervical erosion in women attending a family planning clinic. *Br Med J* 1978; **1:** 748-50.
22. Nayyar KC, O'Neill JJ, Hambling MH, Waugh MA. Isolation of *Chlamydia trachomatis* from women attending a clinic for sexually transmitted diseases. *Br J Vener Dis* 1976; **52:** 396-98.
23. Oriel JD, Johnson AL, Barlow D, Thomas BJ, Nayyar K, Reeve P. Infection of the uterine cervix with *Chlamydia trachomatis. J Infect Dis* 1978; **137:** 443-51.
24. Harkness AH. The pathology of gonorrhoea. *Br J Vener Dis* 1948; **24:** 137-47.
25. Bushell AC, Hobson D. Effect of cortisol on the growth of *C trachomatis* in McCoy cells. *Infect Immun* 1978; **21:** 946-53.
26. Wentworth BB, Bonin P, Holmes KK, Gutman L, Wiesner P, Alexander ER. Isolation of viruses, bacteria and other organisms from venereal disease clinic patients: methodology and problems associated with multiple isolations. *Health Lab Sci* 1973; **10:** 75-81.
27. Mårdh P-A, Ripa KT, Wang S-P, Weström L. *C trachomatis* as an etiological agent in acute salpingitis. In: Hobson D, Holmes KK, (eds). *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 77-83.

British Journal of Venereal Diseases, 1980, 56, 46-48

Urine culture for the detection of *Trichomonas vaginalis* in men

AILSA WILSON AND J P ACKERS

From the Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, London

SUMMARY Urine samples were collected from 248 men, 21 of whom were known contacts of women infected with *Trichomonas vaginalis.* This organism was cultured from only three of the 21 specimens from patients in the contact group. The cultural technique was shown to be capable of reliably detecting small numbers of organisms under practical conditions; it appears, therefore, that most male contacts shed relatively few trichomonads and that the infective dose for women must be correspondingly small.

Introduction

Although most cases of trichomoniasis are believed to be sexually transmitted, demonstration of this organism in men has always been difficult. Here we report a small study designed to assess the value of urine culture in the diagnosis of this infection. We have also attempted to estimate the sensitivity of the method.

Patients and methods

SPECIMENS

Samples of urine were collected from men attending James Pringle House, Middlesex Hospital, during a six-week period in the spring of 1978. Samples were taken from all men except known homosexuals and those already undergoing any kind of treatment. During, and for two weeks before, the study contact slips were issued to all women diagnosed as having trichomoniasis in an attempt to increase the number of male contacts in the study. Samples (20 ml) were stored at room temperature for no longer than four hours before being processed. After centrifugation (500 × *g* for 10 min) the sediment was inoculated into 5 ml of modified Lumsden's medium[1] and incubated for 14 days at 37°C. Cultures were examined by wet-film microscopy after any colour change and also on the fourteenth day of incubation before being discarded.

MEDIA

Three media were used: modified Lumsden's medium;[1] Robinson's medium;[2] and a modification of Bushby's medium.[3] The latter contained (in 2 litres) Tryptone (Difco), 60 g; D-glucose, 40 g; liver infusion (Difco), 36 g; and calcium pantothenate (0·5% solution), 2 ml. After filtering and autoclaving, the following additions were made: sterile inactivated horse serum, 500 ml; cysteine hydrochloride (sterile 30% solution), 10 ml; and chloramphenicol (1% solution), 25 ml.

For the enumeration of small numbers of organisms, pour plates were prepared and incubated as described by Hollander.[4]

DETERMINATION OF MINIMUM INOCULUM

A cryopreserved stock of *T vaginalis* (LUMP 873) was opened and grown in modified Lumsden's medium. Organisms were harvested by centrifugation (at 500 × *g;* for 5 min), washed once in phosphate-buffered saline (pH 7·2; PBS), and resuspended in PBS. After counting in a haemocytameter, dilutions were made in PBS to give 1 to 10^5 organisms in 0·1 ml. Five-millilitre samples of medium were inoculated with 0·1 ml in quintuplicate and incubated for 14 days at 37°C. The number of organisms necessary to infect half of the bottles (ID_{50}) was calculated from the number becoming positive by the method of Reed and Meunch.[5]

DETERMINATION OF SENSITIVITY

To test the sensitivity of the urine-culture method, *T vaginalis* organisms prepared as above were added to 20-ml samples of fresh urine from men previously shown to be free from *T vaginalis.* The number of

Address for reprints: Dr J P Ackers, Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT

Received for publication 14 May 1979

organisms used ranged from 800 to 13 (doubling dilutions). After the urine samples had been inoculated, they were held at room temperature for four hours and then treated exactly as the specimens from the clinic. Cultured *T vaginalis* organisms were also added to urine from men, kept at room temperature, and examined microscopically at intervals. Motility was not significantly reduced after standing for up to 24 hours.

Results

Since the number of organisms present was expected to be low, a preliminary experiment was conducted to determine the best medium for growing *T vaginalis* from small inocula. Using organisms grown in modified Lumsden's medium, known numbers were added to 5-ml quantities of different media; modified Lumsden's and modified Bushby's media both yielded 5/5 positive when inoculated with 10 organisms whereas a much larger inoculum was necessary with Robinson's medium (the ID_{50} was 10 organisms but 10^4 organisms were necessary to give 100% positive cultures). Modified Lumsden's medium was therefore used for urine culture in the remainder of this work.

STUDY POPULATION

Two hundred and eighty-four urine samples were obtained during the study period. Thirty-six patients were excluded from the study because their last known contacts were men, their records were incomplete, or treatment had already begun. Of the remaining 248, 21 were contacts of women diagnosed as having trichomoniasis. One hundred contact slips were issued to women with trichomoniasis during the eight weeks before the study and during the study; as a result, nine men attended and are included in the figure of 21 given above.

CULTURES

T vaginalis was grown from three (14%) of the 21 samples from contacts; in 10 samples, however, bacterial growth was very rapid despite the presence of antibiotics, and it is possible that more samples would have grown *T vaginalis* if this had not occurred. None of the samples from men not known to have been in contact yielded a positive culture, giving an overall success rate of three (1·2%) out of 248 samples.

SENSITIVITY OF TECHNIQUE

In view of these very low results, an attempt was made to assess the sensitivity of the whole technique. Varying numbers of cultured trichomonads were added to 20-ml urine samples and treated exactly as the study samples. The accumulated results of the three separate experiments (see Table) show that fewer than 50 organisms in 20 ml of urine are unlikely to be detected but that 200 or 400 probably will be; the ID_{50} is 70 organisms. To provide a check on the counting and diluting techniques, samples of the four lowest dilutions were added to pour plates of Hollander's medium; the number of colonies produced is also shown in the Table. The numbers confirm the accuracy of the calculated number of organisms being added to the urine.

TABLE *Accumulated results of three experiments to determine sensitivity of urine-culture technique*

No of T vaginalis organisms added to urine	*Cultures becoming positive*		*Mean No of colonies/plate*	
	No	*%*	*No*	*%*
800	2/2	100	ND	
400	2/3	67	ND	
200	6/7	86	ND	
100	7/11	64	68	68
50	8/8	100	30	60
25	1/8	13	14	56
13	2/14	14	10	80

ND = not done

Discussion

We have shown here that our frequent failures to cultivate *T vaginalis* from male urine is not due to a lack of sensitivity of the method. Lumsden *et al*[6] showed that growth could be initiated from about six organisms, although some media require much larger numbers,[7] and we have confirmed that adding 10 or more trichomonads to 5 ml of medium invariably gives a positive result. More realistically, the results suggest that, with a 20-ml urine sample containing more than 200 *T vaginalis* organisms treated by the procedure described, there is an approximately 90% chance of obtaining a positive culture result. The organisms used in these in-vitro studies were grown in fluid medium and therefore might not represent the true behaviour of organisms taken directly from the patient. On the other hand, they were only on their third subculture since isolation and so any changes were probably minimal. Subject to this proviso, therefore, the fact that only 14% (3 out of 21) of samples from known contacts yielded positive culture results implies that the urine of these men contains few viable organisms. An earlier study[8] using identical methods yielded positive culture results from 6/37 (16%) of urine and 6/41 (15%) of semen samples from known male contacts. If the method is as sensitive with semen as with urine samples, most male contacts produce semen which contains only a small number of trichomonads; the infective dose for women, therefore, would also appear to be small.

In previous studies of trichomoniasis in male contacts of infected women, Schapira[9] found 28% positivity by culturing semen samples whereas Summers and Ford[10] found a remarkable 71% positivity using Papanicolaou staining of prostatic fluid and urine deposits (although this method has been criticised).[11] Watt and Jennison[12] obtained 55% positivity from urine cultures in a group of men whose wives had trichomoniasis; in this case, however, the women had long-standing and chronic infections. Our results suggest that, possibly because infected women are seen and cured more rapidly now, urine culture (even in the case of known male contacts) is not likely to lead to many positive results and that it is unlikely to be worth applying routinely to all men who attend.

This work was partly supported by a project grant from the Medical Research Council. We are grateful to Dr R D Catterall and all his staff at James Pringle House for their help in making this study possible.

References

1. Ackers JP, Lumsden WHR, Catterall RD, Coyle R. Antitrichomonal antibody in the vaginal secretions of women infected with *Trichomonas vaginalis*. *Br J Vener Dis* 1975; **51:** 319-23.
2. Robinson GL. The laboratory diagnosis of human parasitic amoebae. *Trans R Soc Trop Med* 1968; **62:** 285-94.
3. Bushby SRM, Copp FC. The antitrichomanal activity of amidonitrothiazoles. *J Pharm Pharmacol* 1955; **7:** 112-7.
4. Hollander DH. Colonial morphology of *Trichomonas vaginalis* in agar. *J Parasitol* 1976; **62:** 826-8.
5. Reed LJ, Muench H. A simple method of estimating fifty percent end points. *Amer J Hyg* 1938; **27:** 493-7.
6. Lumsden WHR, Robertson DHH, McNeillage GJC. Isolation, cultivation, low-temperature preservation and infectivity titration of *Trichomonas vaginalis*. *Br J Vener Dis* 1966; **42:** 145-54.
7. Rayner CFA. Comparison of culture media for the growth of *Trichomonas vaginalis*. *Br J Vener Dis* 1968; **44:** 63-6.
8. Ackers JP, Catterall RD, Lumsden WHR, McMillan A. Absence of detectable local antibody in genitourinary tract secretions of male contacts of women infected with *Trichomonas vaginalis*. *Br J Vener Dis* 1978; **54:** 168-71.
9. Shapira HE. Studies on metronidazole (Flagyl) in the therapy of urogenital trichomoniasis in the male patient. *J Urol* 1965; **93:** 303-6.
10. Summers JL, Ford ML. The Papanicolaou smear as a diagnostic tool in male trichomoniasis. *J Urol* 1972; **107:** 840-2.
11. Perl G. Errors in the diagnosis of *Trichomonas vaginalis* infection as observed among 1199 patients. *Obstet Gynecol* 1972; **39:** 7-9.
12. Watt L, Jennison RF. Incidence of *Trichomonas vaginalis* in marital partners. *Br J Vener Dis* 1960; **36:** 163-6.

British Journal of Venereal Diseases, 1980, **56**, 49-53

Herpesvirus hominis type 2 infection in Ibadan

Problem of non-venereal transmission

D MONTEFIORE, A O SOGBETUN, AND C N ANONG
From the Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria

SUMMARY Examination of sera from blood donors, from patients attending a special treatment clinic, a family planning clinic, and an antenatal clinic showed that the prevalence of herpesvirus hominis type 2 antibodies among the adult population in Ibadan is similar to that in other parts of the world.

The possibility of non-venereal transmission of herpesvirus infection was confirmed by the finding that herpesvirus hominis type 2 could survive on cloth samples under humid tropical conditions for long enough to allow transmission of infection via fomites.

Introduction

In a previous paper[1] we reported on the acquisition of antibodies to type 1 and type 2 herpesvirus hominis (HVH-1 and HVH-2) by children and young adults in Ibadan, Nigeria. Antibodies to HVH-1 were acquired rapidly from the age of 1 year onwards, so that by the time the children had reached teenage approximately 80% had developed antibodies to this type of virus. We suggested that the rapid appearance of HVH-1 antibodies reflected the generally poor living conditions and low socioeconomic status of the population studied.[2]

Antibodies to HVH-2 are uncommon, although not unknown, before the age of puberty,[3] but in Ibadan we found that children started to acquire this type of antibody between the ages of 3 and 5 years. From 5-20 years, which was the oldest age group studied, the proportion of children with HVH-2 antibodies remained virtually constant at about 12% of the population. We suggested that non-venereal transmission of infection could be responsible for this early development of HVH-2 antibodies and that the virus might be capable of remaining viable outside the human body for some hours under humid tropical conditions, thus facilitating possible transfer of infection via fomites. The survival of *Neisseria gonorrhoeae* under such conditions had previously been demonstrated in this laboratory[4] and could possibly account for the relatively common occurrence of gonococcal vulvovaginitis among young girls in Ibadan.[5]

The possible importance of tropical conditions in influencing the spread of infection has also been recently suggested by Leiker,[6] who showed that *Mycobacterium leprae* could remain viable for prolonged periods in the environment under humid tropical conditions but not in the drier, cooler climate of temperate countries.

Our previous study[1] did not include subjects in older age groups and comprised only school pupils or children who had not yet reached school age. All the subjects were therefore either prepubertal or subject to social constraints which were likely to limit to some extent the chance of acquiring disease by sexual contact.

We now report our findings with regards to older subjects, who were either unemployed, or engaged in civilian jobs or in the armed Forces, or were housewives or small traders. We also report the results of experiments which demonstrate that under suitable circumstances type 2 herpesvirus hominis can remain viable outside the human body for long enough to allow the spread of infection via fomites rather than by direct bodily contact.

Patients and methods

ANTIBODY STUDIES

Four groups of subjects were studied.

Blood donors

Blood donors were all male volunteers and included relatives of patients undergoing surgery in the hospital as well as members of the army, police, and civilian youth organisations. A total of 228 subjects was included in this group.

Address for reprints: Professor D Montefiore, Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria

Received for publication 16 July 1979

A sample of blood was collected into a sterile bottle during the blood donation and after clotting had taken place the serum was separated and stored at −20°C until tested.

Many people did not know their age accurately but from the information given the mean age of this group was 28·5 years with a standard deviation (SD) of 6·9 years.

Antenatal clinic patients
Serum samples were obtained from 283 patients attending the Antenatal Clinic. These were initially collected for routine Venereal Disease Research Laboratory (VDRL) tests but were also examined for antibodies to type 1 and type 2 herpesvirus hominis. All samples were stored at −20°C before examination. Individual ages were not available for this group of patients, who were all young women of child-bearing age.

Family planning clinic patients
Serum samples were obtained from 159 patients attending the Family Planning Clinic. Similarly, the samples were originally collected for routine testing by the VDRL test but were also examined for herpesvirus antibodies. Individual ages were not available but all the patients were young women of child-bearing age.

Special treatment clinic patients
Serum samples collected for routine VDRL tests from 167 consecutive patients attending the Special Treatment Clinic were also examined for antibodies to type 1 and type 2 herpesvirus hominis. Of these patients, 125 were men and 42 were women, a sex distribution which reflects the difficulty experienced in contact-tracing and ensuring the attendance of consorts. Although individual ages are unlikely to be accurate, the calculated mean age for this group was 27·8 years with a standard deviation of 8·1 years.

NEUTRALISATION TESTS
The method used for testing the sera has been described previously[1] and was based on that of Pauls and Dowdle[7] with the modifications described by Roome *et al.*[8]

Control sera from rabbits initially immunised against HVH-1 and HVH-2 respectively were included in each batch of tests. These control sera were first tested 78 times against each type of virus, the pN values determined, and the pN-type-2:pN-type-1 ratio calculated. This ratio, multiplied by 100, was termed the NpN value. For the type 1 antiserum the mean NpN value was 78·5, whereas for the type 2 antiserum the mean NpN value was 113·2 with standard deviations of 6·5 and 15·2 respectively. In interpreting the results of test sera those which gave NpN values identical with those of the control sera (±1 SD) were regarded as containing only a single antibody of the appropriate type. Sera with NpN values falling between the control values (±1 SD) were considered to contain both types of antibody.

SURVIVAL OF HERPESVIRUS HOMINIS TYPE 2
Fifteen squares of washed and sterilised white cotton cloth were each inoculated with 0·2 ml of a suspension of herpesvirus hominis type 2 virus in baby hamster kidney (BHK) growth medium containing 10% foetal calf serum to simulate the amount of protein that might be present in an exudate from a herpetic lesion. This volume of viral suspension was sufficient to moisten the cloth samples without leaving any excess fluid.

The inoculated pieces of cloth were placed in sterile Petri dishes and left fully exposed to the air in a room without air-conditioning but shielded from direct sunlight. Wet and dry bulb temperatures were recorded.

At the start of the experiment and thereafter hourly for four hours three cloth samples were eluted in 2 ml of BHK growth medium containing 10 mmol HEPES buffer, 10% foetal calf serum, 0·06% sodium bicarbonate, and antibiotics. Elution was performed by thorough mixing on a Vortex Rotamixer followed by squeezing the fluid from the cloth with sterile forceps.

Each eluate was titrated in triplicate using the BHK-HEPES growth medium as diluting fluid; 0·025 ml of each dilution was placed in the appropriate well of a flat-bottomed Microtitre tissue culture plate, to which 0·05 ml of growth medium was added followed by 0·025 ml of cell suspension in growth medium containing 120 million BHK-21 cells per ml of suspension.

The plates were then sealed, incubated at 35°C in air, and observed daily for five days for the development of cytopathic effect. At the end of the observation period viral titres were calculated and expressed as viral content per cloth sample.

EVALUATION OF RESULTS
Where appropriate, χ^2 and P values were calculated using the Hewlett-Packard HP 65 calculator.

Results

ANTIBODY STUDIES
The results of the antibody studies are shown in Table 1. Nearly all these adult subjects had antibodies to either one or both viral types. In the blood donor group (consisting only of men), where individual ages were known, the mean age of those

TABLE I *Results of antibody studies to herpesvirus hominis among adults in Ibadan*

Patient group	*Total tested (A+B+C+D)*	*Negative (A)**		*Type 1 antibody only (B)*		*Type 2 antibody only (C)*		*Types 1 and 2 antibody (D)*		*Total with type 1 antibody (B+D)*		*Total with type 2 antibody (C+D)*	
		No	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*
Blood donors	228	6	2·6	175	76·7	24	10·5	23	10·1	198	86·8	47	20·6
Antenatal Clinic patients	283	2	0·7	202	71·4	21	7·4	58	20·5	260	91·9	79	27·9
Family Planning Clinic patients	159	2	1·3	113	71·1	10	6·3	34	21·4	147	92·5	44	27·7
Special Treatment Clinic patients	167	1	0·6	109	65·3	14	8·4	43	25·7	152	91·0	57	34·1

*Sera containing no detectable antibody at lowest serum dilution (1/10) tested

without type 2 antibody was 28·7 years (SD 6·8) whereas the mean age of those with type 2 antibodies was 29·1 years (SD 7·5). Similarly, the prevalence of type 2 antibodies did not appear to be related to either sex or age in the group of patients attending the Special Treatment Clinic. Individual ages were not available for the groups of female subjects attending either the Antenatal Clinic or the Family Planning Clinic.

A high proportion of subjects in each of the groups possessed antibodies against type 1 virus. The proportions do not differ significantly between the groups, with $\chi^2_1=3{\cdot}05$, $P=0{\cdot}08$ when blood donors are compared with the Family Planning group, $\chi^2_1=3{\cdot}44$, $P=0{\cdot}06$ when compared with the Antenatal Clinic subjects, and $\chi^2_1=1{\cdot}67$, $P=0{\cdot}2$ when compared with Special Treatment Clinic patients.

The proportion of blood donors with type 2 antibodies was however smaller than that found in the other groups. Comparison between the proportion of blood donors with type 2 antibodies and those attending the Special Treatment Clinic yields a χ^2_1 value of 9·08, $P=0{\cdot}003$. Although the difference between the proportion of blood donors with type 2 antibody and the proportion of positive subjects attending either the Antenatal Clinic ($\chi^2_1=3{\cdot}62$, $P=0{\cdot}06$) or the Family Planning Clinic ($\chi^2_1=2{\cdot}60$, $P=0{\cdot}11$) is not statistically significant when each of these are considered separately, it does reach the generally accepted level of significance if the numbers are increased by combining the very similar results for the latter two groups ($\chi^2_1=4{\cdot}13$, $P=0{\cdot}04$).

SURVIVAL OF HERPESVIRUS HOMINIS TYPE 2

The viral titres are given in Table 2.

TABLE II *Viral content of cloth samples* after exposure to room conditions† for varying times*

Time (hours)	*Log$_{10}$ titre*			
	Sample 1	*Sample 2*	*Sample 3*	*Mean*
0	4·0	4·1	4·1	4·06
1	2·8	2·0	2·6	2·48
2	1·5	1·3	0·0	0·93
3	1·1	0·0	0·0	0·36
4	0·0	0·0	0·0	0·0

*Mean of three titrations for each sample
†Dry bulb temperature 31·1°C, wet bulb temperature 30°C, relative humidity 83%

At the temperature (31·1°C dry bulb) and relative humidity (83%) at which the experiment was conducted, the cloth samples were still clearly moist after one hour. Definite drying was apparent by the second hour and the cloth samples were dry to visual inspection by the third hour. At night, when the relative humidity increases to nearly 100%, these drying times could be considerably prolonged.

The viral titre fell by approximately 1·5 log units during each of the first two hours and little virus was detectable by the third hour. No virus could be isolated by the fourth hour, when the cloth samples were apparently completely dry.

Discussion

We have previously reported[1] that by the age of 20 years some 80% of the Ibadan population possesses antibody to herpesvirus hominis type 1, and the present results show that this proportion increases to nearly 90% in subjects who are about 10 years older. We also found that by the age of 20 years some 12% of the population had developed antibodies to type 2 virus; this study shows that this proportion also increases among older subjects.

Approximately 20% of blood donors in Ibadan had antibodies to HVH-2—a prevalence that is almost identical to that reported earlier by Adelusi *et al*[9] for a group of asymptomatic control subjects in Ibadan. It is also very similar to that reported by Roome *et al*,[8] who examined blood donors in Bristol

(England) and found that approximately 18% of them possessed antibodies to HVH-2.

In the present study type 2 antibodies were present in a higher proportion of patients attending the Antenatal and Family Planning Clinics but the prevalence is very similar to that found among asymptomatic pregnant women in America.[10] The highest prevalence of type 2 antibodies was found among patients attending the Special Treatment Clinic, and this too agrees with the widely accepted finding that genital herpetic infections occur most frequently among patients attending venereal diseases clinics[11] and among those with the greatest sexual exposure.[12]

Our findings relating to adult groups of patients are thus not at variance with those of other workers and would accord with the accepted view that type 2 herpesvirus hominis is generally sexually transmitted—a view that is based not only on sero-epidemiological data but also on the findings that type 2 virus is isolated from most genital herpetic lesions and that genital herpes is often associated with the presence of other infections known to be sexually transmitted.[13 14]

However, our previous report[1] that antibodies to HVH-2 appear in early childhood is unusual. It seems most unlikely that some 11% of 5-year-old children in Ibadan could have been in close sexual contact with individuals with active genital herpes or even in non-sexual contact since most HVH-2 lesions occur around the genitalia, thighs, and buttocks.[15] Nor would the appearance of HVH-2 antibodies at this age be likely to result from a serological cross-reaction with varicella antibodies, although childhood chicken-pox is common, in view of previous findings by Gerna *et al*[16 17] that the sensitive immunoperoxidase method failed to detect group cross-reactions at serum dilutions greater than 1/10 to 1/16.

Although herpesvirus transmission is generally considered to require close bodily contact, the possibility of transmission via fomites has to be taken into account. Herpesvirus hominis is regarded as a fairly labile virus,[18 19] but its rate of inactivation is known to vary considerably under different environmental conditions. When drying is prevented by transport media, the virus can survive for several days at room temperature with little loss of titre,[20 21] and it has also been found to survive for some weeks at room temperature in dried crusts from herpetic lesions.[22]

There have been reports that genital herpes may sometimes be acquired by non-venereal means. Indeed, as early as 1940, when the differences between HVH-1 and HVH-2 were unknown, Sharlit[23] wrote that, ". . . none will be so rash as to challenge the claim of celibacy or to impugn the professions of chastity or fidelity on the prima facie evidence of herpes progenitalis."

Childhood genital herpes has been recorded on several occasions, although most reports were published before the two types of herpes viruses had been differentiated. Brain[24] described acute herpetic vulvovaginitis of infant girls in 1956 and suggested that the virus might enter through skin lesions, such as napkin rashes, wounds, burns, or abrasions. Krugman[25] and Scott *et al*[26] had both previously reported cases of genital herpes in children but again the type of virus is not known. Sheward[27] later reported perianal herpes in twins aged 2 years. Circumstantial evidence suggests that in these cases the infection was probably due to type 1 virus, possibly transmitted by the zinc and castor oil cream which was applied from the same container to both the infants' napkin rash and their father's "cold sores." Evidence suggesting that at least HVH-1 may be transmitted by fomites has also been supplied by Stern *et al*[28] and by Rosato *et al*[29], who found herpetic whitlow among medical personnel, in whom many cases may occur without known direct contact with oropharyngeal secretions from patients.

Some years ago, Nahmias *et al*[30] described six cases of genital herpes in young children, five of whom were girls and one a boy. Four of the girls were infected with HVH-2 whereas the other children were infected with HVH-1. Two of the girls with HVH-2 infection denied any sexual contact, although the authors of the report considered that the infection must have been sexually acquired.

Amstey and Balduzzi,[31] however in an account of 15 patients with genital herpes, mentioned one girl who was 15 years old, a virgin, and denied sexual contact. The authors commented that herpesvirus infections of the female genitalia may not be transmitted exclusively by sexual means. This view was reiterated by Kessler[32] when reporting the unexpected finding that 58·8% of a group of nuns possessed antibodies to HVH-2 whereas 57·4% of their blood sisters, who were not nuns, also had antibodies to the virus. The latter group however frequently had trichomonads in their vaginal secretions, indicating sexual exposure, whereas no trichomonads were detected among the nuns. Roome *et al*[8] also suggested that non-venereal spread of HVH-2, possibly by damp towels, might explain the very high incidence of HVH-2 antibodies which they detected among long-term prison inmates.

There are therefore grounds for suggesting that not all HVH-2 infection is sexually acquired, and our present findings indicate that in the tropics the virus could survive outside the human host for sufficient time to allow infection to be transmitted via fomites, such as shared bed-clothes, towels, or underclothing. If this is so, many of the lesions are likely to be mild and insufficiently troublesome for the child to be brought for medical attention under conditions where medical facilities are scarce and home-treatment of self-limiting conditions common. By the time that children in Ibadan start to develop HVH-2 antibodies, over 50% have already been exposed to HVH-1; the cross-immunity acquired from these infections would substantially reduce the severity of the illness after subsequent exposure to HVH-2.[33]

We would like to thank the Wellcome Trust for a grant towards the cost of this study.

References

1. Sogbetun, AO, Montefiore, D, Anong CN. Herpesvirus hominis antibodies among children and young adults in Ibadan. *Br J Vener Dis* 1979; **55:** 44-7.
2. Smith IW, Peutherer JF, MacCallum FO. The incidence of herpesvirus hominis antibody in the population. *J Hyg (Camb)* 1967; **65:** 395-408.
3. Nahmias AJ, Josey W, Naib ZM, Luce CF, Duffey A. Antibodies to herpesvirus hominis type 1 and 2 in humans. I Patients with genital herpetic infection. *Am J Epidemiol* 1970; **91:** 539-46.
4. Alausa KO, Sogbetun AO, Montefiore D. Effect of drying of *Neisseria gonorrhoeae* in relation to non-venereal infection of children. *Niger J Paediatr* 1977; **4:** 14-8.
5. Osoba, AO, Alausa KO. Vulvovaginitis in Nigerian children. *Niger J Paediatr* 1974; **1:** 26-32.
6. Leiker DL. On the mode of transmission of *Mycobacterium leprae*. *Lepr Rev* 1977; **48:** 9-16.
7. Pauls FP, Dowdle WR. A serological study of herpesvirus hominis strains by microneutralisation tests. *J Immunol* 1967; **98:** 941-7.
8. Roome APCH, Montefiore D, Waller D. Incidence of herpesvirus hominis antibodies among blood donor populations. *Br J Vener Dis* 1975; **51:** 324-8.
9. Adelusi B, Osunkoya BO, Fabiyi A. Herpes type 2 virus antibody studies in groups of patients with neoplasms in Ibadan. *Afr J Med Sci* 1976; **5:** 297-301.
10. Polognese RJ, Corson SL, Fuccillo DA, Traub R, Moder F, Sever JL. Herpesvirus hominis type 2 infections in asymptomatic pregnant women. *Obstet Gynecol* 1976; **48:** 507-10.
11. Willmott FE, Mair HJ. Genital herpes virus infection in women attending a venereal diseases clinic. *Br J Vener Dis* 1978; **54:** 341-3.
12. Duenas A, Adam E, Melnick JL, Rawls WE. Herpesvirus type 2 in a prostitute population. *Amer J Epidemiol* 1972; **95:** 483-9.
13. Wolontis S, Jeanson S. Correlation of herpes simplex virus types 1 and 2 with clinical features of infection. *J Infect Dis* 1977; **135:** 28-33.
14. Jeanson S, Molin L. Genital herpes infection and non-specific urethritis. *Br Med J* 1971; **3:** 247.
15. Nahmias AJ, Roizman B. Infection with herpes simplex virus types 1 and 2. *New Engl J Med* 1973; **289:** 781-9.
16. Gerna G, Chambers RW. Varicella-zoster plaque assay and plaque reduction neutralisation test by the immuno-peroxidase technique. *J Clin Path* 1976; **4:** 437-42.
17. Gerna G, Achilli G, Chambers RW. Determination of neutralising antibody and IgG antibody to varicella zoster virus and IgG antibody to membrane antigens by the immunoperoxidase technique. *J Infect Dis* 1977; **135:** 975-9.
18. Scott TFM, McLeod DL, Tokumaru, T. A biologic comparison of two strains of herpesvirus hominis. *J Immunol* 1961; **86:** 1-12.
19. Plummer G, Waner JL, Phuansab A, Goodheart CR. Type 1 and 2 herpes simplex viruses: serological and biological differences. *J Virol* 1970; **5:** 51-9.
20. Roome APCH, Montefiore D, Waller D. Transport media for herpesvirus hominis type 2. *Niger Med J* 1976; **6:** 281-4.
21. Rodin P, Hare MJ, Barwell CF, Withers MJ. Transport of herpes simplex virus in Stuart's medium. *Br J Vener Dis* 1971; **47:** 198-9.
22. Nahmias AJ, Wickliffe C, Pipkin J, Leibovitz A, Hutter R. Transport media for herpes simplex virus types 1 and 2. *Appl Microbiol* 1971; **22:** 451-4.
23. Sharlit H. Herpes progenitalis as a venereal contagion. *Arch Dermatol Syphilol* 1940; **42:** 933-6.
24. Brain RT. The clinical vagaries of herpes virus. *Br Med J* 1956; **1:** 1061-8.
25. Krugman S. Primary herpetic vulvovaginitis. Report of a case: isolation and identification of herpes simplex virus. *Pediatrics* 1952; **9:** 585-8.
26. Scott TFM, Coriell L, Blank H, Burgoon CF. Some comments on herpetic infection in children with special emphasis on unusual manifestations. *J Pediatr* 1952; **41:** 835-43.
27. Sheward JD. Perianal herpes simplex. *Lancet* 1961; **1:** 315-6.
28. Stern H, Elek SD, Millar DM, Anderson HF. Herpetic whitlow: a form of crossinfection in hospitals. *Lancet* 1959; **2:** 871-4.
29. Rosato FE, Rosato EF, Plotkin SA. Herpetic paronychia—an occupational hazard of medical personnel. *New Engl J Med* 1970; **283:** 804-5.
30. Nahmias AJ, Dowdle WR, Naib ZM, Josey WE, Luce CF. Genital infection with herpesvirus hominis type 1 and 2 in children. *Pediatrics* 1968; **42:** 659-66.
31. Amstey MS, Balduzzi PC. Genital herpesvirus infection. *Am J Obstet Gynecol* 1970; **108:** 188-93.
32. Kessler II. Human cervical cancer as a venereal disease. *Cancer Res* 1976; **36:** 783-91.
33. Josey WE, Nahmias, AJ, Naib ZM. The epidemiology of type 2 (genital) herpes simplex virus infection. *Obstet Gynecol Surv* 1972; **27:** 295-302.

British Journal of Venereal Diseases, 1980, **56**, 54-56

Sexually transmitted diseases in clinic patients in Lagos

V O ROTIMI AND A O SOMORIN

From the Departments of Microbiology and Parasitology and Medicine, Lagos University Teaching Hospital, Lagos, Nigeria

SUMMARY In a study of patients attending an STD referral clinic in Lagos, Nigeria, the age distribution was similar to that seen in other parts of the world and the male-to-female ratio was 1·3:1. Non-specific genital infection was diagnosed in about 59% of patients and gonorrhoea in 19%. Most strains of *Neisseria gonorrhoeae* were sensitive to penicillin (1 unit) and the six relatively penicillin-resistant strains did not produce penicillinase. Candidosis and trichomoniasis were each diagnosed in about 10% of patients and latent syphilis in 1·4% (four cases). Genital warts were also uncommon; venereophobia and other conditions accounted for 1% and 2·2% of cases respectively.

Introduction

There is general concern at the worldwide increase in sexually transmitted diseases (STDs). A review of the global prevalence of gonorrhoea described the numbers as "of epidemic proportions."[1] In Nigeria, as in many African countries, accurate statistics on STDs are not readily available. However, STDs, particularly gonorrhoea, are at least as common in Africa as in other parts of the world.

Lagos is the federal capital of Nigeria and has a population of about 1·5 million; it is the principal commercial and industrial centre of the country. As in western countries, casual sexual relationships with the attendant risk of STDs are common. Treatment is often unsatisfactory and complications such as urethral stricture and "watering-can" perineum are still common in Nigeria.[2] There is an established aetiological association among Nigerians between hypertension and post-gonococcal urethral stricture.[3] The gonococcus is an important cause of infertility or subfertility in developing countries.[4]

Lagos has only one specialised centre for the adequate investigation and treatment of STDs, which serves the greater part of the city and its surroundings. The acute shortage of doctors in the country forces some patients to seek treatment from private medical practitioners, often at exorbitant prices, while others consult pharmacists, native doctors, divine healers, and quacks.

We report our experience of STDs seen in the outpatient population attending Lagos University Teaching Hospital STD clinic over a period of 15 months from October 1976 to December 1977.

Patients and methods

STUDY POPULATION

The study population comprised 276 patients who were either referred to the STD clinic or invited to attend through contact-tracing between October 1976 and December 1977. The clinic was manned by a consultant venereologist/dermatologist, a microbiologist (trainee), a senior technician, two nursing sisters, two social workers, and one aide.

CULTURAL TECHNIQUES AND SEROLOGICAL TESTS

Urethral specimens were collected from all men who presented with urethral discharge using a sterile cotton-wool swab passed into the anterior urethra and gently rotated; the swab was plated directly on to plates of Thayer-Martin (Oxoid) and chocolate agar medium. The plates were incubated immediately in a candle jar at 37°C for 24 and 48 hours. Smears made from the swabs were Gram-stained and examined for intracellular Gram-negative diplococci and leucocytes. In women specimens were obtained with a swab from the cervical os and vaginal vault under direct vision with a Cusco's speculum and processed as above. A two-glass urine test was performed on the urine specimen from all men and the centrifuged deposit from each specimen showing threads or specks was examined microscopically for leucocytes,

Address for reprints: Dr V O Rotimi, Department of Medical Microbiology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2 RX

Received for publication 18 February 1979

red blood cells, and organisms. All Gram-negative cocci growing on chocolate agar or Thayer-Martin agar were subcultured on to fresh Thayer-Martin agar and identified by the oxidase reaction and fermentation reactions in 1% sugar media.[5] Isolates which were oxidase-positive and fermented glucose but not maltose or sucrose were identified as *Neisseria gonorrhoeae.*[6]

A wet preparation of the genital discharge was examined for *Trichomonas vaginalis, Candida albicans,* and parasites, and specimens showing yeast cells were plated on Sabouraud's agar; *C albicans* was identified by the germ-tube test.

Serum from each patient was tested by the Venereal Disease Research Laboratory (VDRL) test and Reiter protein complement fixation test (RPCFT). Where indicated, the Frei test and the lymphogranuloma venereum complement fixation test (LGVCFT) were also performed.

Gram-stained smears from ulcers were examined for *Haemophilus ducreyi.*

MIC DETERMINATIONS FOR *N GONORRHOEAE*

The six strains of *N gonorrhoeae* isolated from the patients in Lagos that were found to be relatively resistant to penicillin (1 unit disc) were lyophilised and reidentified; the minimum inhibitory concentration (MIC) of penicillin was determined in the UK by one of us (VOR) with the following international reference strains of gonococci as controls; strains K2, V, and III (originally from Alice Reyn's laboratory).

The determination of the MIC of penicillin for the six resistant strains from Lagos was performed by method already described[7] and a reference strain of *Staphylococcus aureus* (Oxford) was included with each batch of test strains.

Penicillinase production

Penicillinase production was detected by the chromogenic cephalosporin (nitrocefin) method[8] and the starch paper technique.[9]

Results

SEX RATIO AND AGE

Two hundred and seventy-six patients were seen during this study; 156 (57%) were men and 120 (43%) women, a male-to-female ratio of 1·3:1. Although 43% (119) of the patients were aged between 16 and 29 years (Table I), very few were in the lower part of this age range—probably because most of the young patients were treated privately.

TABLE I *Age distribution of clinic patients*

Age (years)	*Men*	*Women*	*Total*	
			No	*%*
16-20	8	3	11	4·0
21-24	52	21	73	26·4
25-29	27	8	35	12·7
30-34	13	10	23	8·4
35-40	6	15	21	7·6
>40	4	16	20	7·2
Uncertain	46	47	93	33·7

TABLE II *Analysis of conditions diagnosed*

Diagnosis	*No*	*%*
Non-specific genital infection	164	59·4
Gonorrhoea	53	19·2
Candidosis	29	10·5
Trichomoniasis	29	10·5
Lymphogranuloma venereum	1	0·36
Syphilis	4	1·4
Genital warts	5	1·8
Venereophobia	3	1·08
Others	6	2·2

DIAGNOSES

The most common diagnosis was non-specific genital infection (NSGI); this was made in 164 (59·4%) of the 276 patients (Table II). Gonorrhoea was diagnosed in 53 (19·2%) patients and candidosis and trichomoniasis each in 29 (10·5%).

Antitreponemal antibodies were detected in 1·4% of patients; all were cases of latent syphilis. Venereophobia, lymphogranuloma venereum, genital warts, and a miscellaneous group of conditions accounted for the remainder of cases (Table II). More than one diagnosis was confirmed in 6·5% of patients.

SOURCE OF INFECTION

Analysis of the source of infection (Table III) showed that 34·1% of all patients contracted infection through casual partners whom the patients had met only once and could not name or trace; regular partners and prostitutes were the source of infection in 11·6% and 19·2% respectively.

MIC FOR GONOCOCCAL STRAINS

Four strains of *N gonorrhoeae* had an MIC of 0·075-0·3 μg/ml and two strains an MIC of >0·6 μg/ml.

TABLE III *Source of infection*

Source	*No*	*%*
Regular partner	32	11·6
Casual partner	94	34·1
Prostitute	53	19·2
Spouse	31	11·2
Unknown	66	23·9
Total	276	100·0

β-LACTAMASE PRODUCTION

None of the six strains that were relatively resistant to penicillin produced β-lactamase.

Discussion

About 40% of the patients in the present study were aged between 21 and 29 years, a lower figure than the 51% recorded in Ibadan.[10] This could be because our clinic was a referral clinic and the figure therefore does not give a true picture of the incidence of STD in this age group. Many young people are afraid of attending an STD clinic because of the social stigma and shame associated with STDs and also because of the long hours of waiting in the hospital. Hence many seek private treatment.

Nearly 60% of the patients in this study had NSGI, an incidence similar to that commonly reported in other parts of the world. Patients with NSGI usually presented with a slight discharge, which occasionally was profuse and purulent, together with mild dysuria and frequency in men. Non-specific urethritis, manifested by clinical evidence of urethritis without positive microbiological findings, was reported as the commonest cause of STD in men in England.[11] Reports from elsewhere show that this is probably the commonest cause of STD.[10 12]

The figure of about 20% of gonococcal infections is similar to that found in Ibadan,[10] Uganda,[13] and Singapore.[14] Antibiotics are freely available in Nigeria and there is no action against their indiscriminate sale, so self-medication is very common. The absence of effective legislation to control the sale of antimicrobial drugs has contributed greatly to sexual promiscuity and spread of STDs in this country. It is a popular belief among clinic patients that a capsule of tetracycline will give prophylactic cover against gonorrhoea.

The four cases of syphilis were all latent and diagnosed by positive results to serological tests in the absence of any previous history of yaws. Syphilis was also uncommon in Ibadan[10] and in Kampala.[15] Venereophobia was not frequently seen perhaps because the clinic was not a "walk-in" one; the three cases we did see were referred to the psychiatrist.

Four of the six isolates from Lagos had MICs of 0·075-0·3 μg/ml. These were obviously strains of reduced sensitivity. The remaining two strains were markedly resistant with MICs of >0·6 μg/ml. Earlier reports showed that resistant strains of *N gonorrhoeae* started to appear in 1944.[16] A progressive increase in gonococcal resistance to antibiotics, especially to penicillin, has been reported.[1 17 18] None of the six strains in this study produced β-lactamase. A similar study in Ibadan also failed to demonstrate penicillinase production in a larger number of gonococci.[19]

We are grateful to Professor O Ogunbi for his encouragement and help and to Messrs S F Lawal, and I Nwabudike and Dr Toye Coker for their technical assistance and help. We are indebted to Professor M G McEntegart for reading through this paper and for his valued criticism. We also thank Dr A Hilton and Dr B I Duerden for comments on the manuscript and Mrs Hazel Bland for her secretarial assistance.

References

1. Willcox RR. A world look at venereal diseases. *Med Clin North Am* 1972; **56:** 1057-72.
2. Osoba AO, Alausa O. Gonococcal urethral stricture and watering-can perineum. *Br J Vener Dis* 1976; **52:** 387-93.
3. Elebute EA. Post-gonorrhoeal urethral stricture and hypertension in Nigeria. *Tran R Soc Trop Med Hyg* 1966; **60:** 676-80.
4. Belsey MA. The epidemiology of infertility: a review with particular reference to sub-saharan Africa. *Bull WHO* 1976; **54:** 319-41.
5. Flynn J, Waitkins SA. A serum-free medium for testing fermentation reaction in *N gonorrhoeae. J Clin Path* 1972; **25:** 525-7.
6. Cowan ST. *Manual for the Identification of Medical Bacteria,* 2nd ed. Cambridge University Press, 1974.
7. Ericsson HM, Sherris JC. Antibiotic sensitivity testing. Report of an international collaborative study. *Acta Pathol Microbiol Scand* 1971; Suppl **217:** 14
8. O'Callaghan CH, Morris A, Kirky, SM, Slinger AH. Novel method for detection of β-lactamase by using a chromogenic cephalosporin substrate. *Antimicrob Agents Chemother* 1972; **1:** 283-8.
9. Odugbemi TO, Hafiz S, McEntegart MG. Penicillinase-producing *N gonorrhoeae:* detection by starch paper technique. *Br Med J* 1977; **2:** 500.
10. Sogbetun AO, Alausa KO, Osoba AO. Sexually transmitted diseases in Ibadan, Nigeria. *Br J Vener Dis* 1977; **53:** 155-60.
11. Arya OP, Lawson JB. Sexually transmitted diseases in the tropics. Epidemiological, diagnostic, therapeutic and control aspects. *Trop Doct* 1977; **1:** 57-66.
12. Willcox RR. Importance of the so-called other sexually transmitted diseases. *Br J Vener Dis* 1975; **51:** 221-6.
13. Arya OP, Msanzumulure H, Tuber SR. Clinical, cultural and demographic aspects of gonorrhoea in a rural community in Uganda. *Bull WHO* 1973; **49:** 587.
14. Rajan VS. Sexually transmitted diseases on a tropical island. *Br J Vener Dis* 1978; **54:** 141-3.
15. Ongom VL, Wamboka JW, Nakagwa E, Yigg A, Kamulegeya JK, Munafa C. The prevalence of venereal diseases among food- and liquor-handlers in public places in Kampala, Uganda. *East Afr Med J* 1976; **5** (7): 389-97.
16. Reyn A, Kosner B, Bentzon MW. Effects of penicillin, streptomycin and tetracycline on *N gonorrhoeae* isolated in 1944 and in 1957. *Br J Vener Dis* 1958; **34:** 277-9.
17. Amies CR. Sensitivity of *Neisseria gonorrhoeae* to penicillin and other antibiotics. *Br J Vener Dis* 1969; **45:** 216-22.
18. Willcox RR. A survey of problems in the antibiotic treatment of gonorrhoea with special reference to South East Asia. *Br J Vener Dis* 1970; **46:** 217-42.
19. Osoba AO, Montefiore DG, Sogbetun AO, Alausa KO, Anong CN. Sensitivity pattern of *Neisseria gonorrhoeae* to penicillin and screening for β-lactamase production in Ibadan, Nigeria. *Br J Vener Dis* 1977; **53:** 304-7.

British Journal of Venereal Diseases, 1980, **56,** 57

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Diverticular disease presenting at a special clinic[*]

Sir,

Vesico-colonic fistula is a well known but uncommon complication of diverticulitis,[1] presenting with well-recognised features such as pneumaturia, cystitis, and faecaluria.[2] Presentation with urethral discharge alone however must be very rare.

A 69-year-old, retired merchant seamen attended the Special Clinic of this hospital in November 1977 with a three-week history of dysuria and nocturia without day-time frequency, which were unresponsive to courses of pyridium and cetiprin prescribed by his general practitioner. He had been treated for gonorrhoea 30 years previously and came to the clinic because one week before he had developed a urethral discharge. He denied any sexual intercourse since his treatment for gonorrhoea.

On examination he had a mucopurulent urethral discharge. The urine was hazy with heavily blood-stained threads. A Gram-stained urethral smear showed no evidence of Gram-negative diplococci but numerous leucocytes were seen. Inoculation of mucopus on to modified Thayer-Martin medium failed to produce a growth of *N gonorrhoeae.* Serological tests for syphilis gave negative results. No abnormalities were found on examination of the abdomen or the prostate gland. A presumptive diagnosis of nongonococcal urethritis (NGU) with associated cystitis was made and co-trimoxazole two tablets every 12 hours was prescribed. *Escherichia coli* ($>10^8$/l) were grown on culture of the urine.

Four days later at his next attendance a 10-day history of pneumaturia was obtained and, on further questioning, he admitted to the passage of faeculent material from the urethra. He had had no recent abdominal pain or bowel disorder. A vesico-colonic fistula was diagnosed and the patient referred to the surgical department for further investigation. Barium enema failed to show a fistula but fairly extensive diverticular disease of the sigmoid colon was evident. Excretion urography was normal. Cystoscopy showed a small fistula with faecal material exuding through it. Surgical correction was clearly necessary and a small vesico-colonic fistula was found at operation. After separation of the sigmoid colon from the bladder the defect was closed and a sigmoid colectomy with end-to-end anastomosis performed. Histology showed the sinus to be lined with granulation tissue extending through the muscularis.

E coli is an uncommon pathogen in the aetiology of NGU, but as it was recovered from the urine in this patient it apparently had a pathogenic role in his urethritis. Coliforms passed easily from the bowel causing a urethral discharge, which brought his condition to our attention. At present the patient is symptomfree with sterile urine 11 months after operation. The aetiology of NGU in elderly men obviously requires unbiased consideration.

We should like to thank Dr C D Alergant and Mr C R Helsby for their assistance.

Yours faithfully,

P B Carey
Department of Venereology,
Royal Liverpool Hospital,
Liverpool L7 8XP

Department of Surgery, *J N Johnson*
Royal Liverpool Hospital,
Liverpool L7 8XP

References

1. Henderson MA, Small WP, Vesico-colonic fistula complicating diverticular disease. *Br J Vener Dis* 1969; **41:** 314-9.
2. Krompier A, Howard R, MacEwan R, Natoli C, Wear JB. Vesicocolonic fistulas in diverticulitis. *J Urol* 1976; **115:** 664-6.

Notice

30th General Assembly of the IUVDT

The 30th General Assembly of the International Union against the Venereal Diseases and the Treponematoses will take place from 6 to 12 June 1980 in East Berlin.

There will be five scientific sessions and an extensive social programme. The scientific sessions will be: syphilis, gonorrhoea, viral sexually transmitted diseases, other sexually transmitted diseases, and social/psychological/epidemiological aspects of venereal and other sexually transmitted diseases.

Participants and observers are referred to: Professor G Elste, Chefartz: Medinzalrat Hautklinik, Stadtisches Klinikum, Wiltbergstrasse 50, 115 Berlin-Buch, East Germany.

Third Meeting

Sexually Transmitted Diseases, third meeting, Antwerp, Belgium, from 2 to 3 October 1980. Main topics include: genital infections in women and genital ulcers.

Abstracts to be submitted by 30 May 1980 to the programme secretary: Dr P Piot, Department of Bacteriology and Virology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium.

British Journal of Venereal Diseases, 1980, **56**, 58

Book review

Human Chlamydial Infections. By Julius Schachter and Chandler R Dawson, 1978. Pp 273 PSG Publishing Company Inc, Littleton, Massachusetts, USA.

This book is very welcome. The literature on chlamydial infection is now so enormous that there is a real need for an authoritative review of the role of chlamydia in human disease for clinicians, microbiologists, and public health workers. The authors' emphasis is predominantly clinical, and there are chapters on psittacosis, lymphogranuloma venereum, ocular disease, neonatal infections, genital tract infections, and Reiter's disease. There is little overlap between sections, and although each can be read independently I imagine that most readers will want to read the whole book—a pleasure in any case owing to the authors' mellifluous literary style. Chapters on the microbiology of chlamydia and on laboratory diagnosis (the latter containing enough detail to be of practical value to laboratory workers) conclude the volume.

One of the most valuable features of this book is the discussion at the end of each clinical section of what is known and unknown about the various diseases. One realises how much has yet to be discovered, and it is chastening to be reminded that the main clinical and epidemiological features of chlamydial genital infection were established as long ago as 1911. The authors adopt a balanced and judicious view of controversial matters such as latency in chlamydial infections of the genital tract, the aetiology of nongonococcal urethritis, and the role of chlamydia in Reiter's disease.

The book is well produced and contains an excellent series of colour photographs of ocular infections; in contrast, those of genital infections are rather disappointing. There is a good list of references up to 1977. No doubt physicians and laboratory workers interested in genital tract infections will wish that "their" diseases were covered in even greater detail, but this is outweighed by the obvious advantages of the broad view of the whole field of chlamydial infection which the authors provide. Indeed, such is their skill in presenting a vast amount of knowledge in a succinct and readable form that the absence of a venereologist is hardly noticeable.

J D Oriel

British Journal of Venereal Diseases, 1980, **56**, 59-64

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
  (Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea
  (Clinical; microbiology; therapy)
Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases
Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (clinical and therapy)

Penicillin levels in blood and CSF achieved by treatment of syphilis
EMC DUNLOP, SS AL-EGAILY, AND ET HOUANG (London, England). *JAMA* 1979; **241:** 2538-40.

This paper, from the Whitechapel Clinic, London, reports the results obtained in an investigation to determine what dosages and preparations of penicillin are required to give treponemicidal concentrations of penicillin in the cerebrospinal fluid (CSF). Only penicillin G was used. All the patients had late acquired or congenital syphilis. The samples of CSF were taken after treatment. Assays were performed using a microbiological method with *Sarcina lutea* as the test organism.

Five men were treated with PAM 600 000 IU intramuscularly daily for 10 days. CSF concentrations of penicillin in these patients were below 0·03 IU/ml, which is the minimum concentration recommended by the WHO to achieve a treponemicidal effect. Four men were treated with penicillin G procaine 600 000 IU daily for 10 days, and therapeutic concentrations were not achieved in the CSF. Using penicillin G 500 000 IU i.m. six hourly for 17 days with oral probenecid 500 mg six-hourly or 2·4 megaunits penicillin G procaine daily with oral probenecid 500 mg six-hourly, treponemicidal concentrations were found in all patients.

If penicillin G procaine 600 000 IU daily was combined with probenecid 500 mg six-hourly, treponemicidal concentrations in the CSF were obtained in two patients with normal CSF but not in one patient with a raised protein level. A similar result was achieved when 1·2 megaunits penicillin G procaine was used with oral probenecid; only patients with normal CSFs had treponemicidal concentrations of penicillin.

G D Morrison

Cystoid macular edema as the primary sign of neurosyphilis
NF MARTIN AND CR FITZGERALD (Florida, USA). *Am J Ophthalmol* 1979; **88:** 28-31.

Syphilitic osteomyelitis with diffusely abnormal bone scan
D SIEGEL AND SZ HIRSCHMAN (New York, USA). *Mt Sinai J Med NY* 1979; **46:** 320-2.

Syphilis (serology and biological false-positive phenomenon)

A solid-phase radioimmunoassay for the detection of antibodies to *Treponema pallidum*
JM MILLER, EM DONOWHO, DW TRENT, AND EEM MOODY (Texas, USA). *Sex Trans Dis,* 1979; **6:** 43-9.

Syphilis (pathology and experimental)

Unique lipid composition of *Treponema pallidum* (Nichols virulent strain)
HM MATTHEWS, T-K YANK, AND HM JENKINS (Minnesota, USA). *Infect Immun* 1979; **24:** 713-9.

Electron microscopy of treponemes subjected to the *Treponema pallidum* immobilization (TPI) test
K HOVIND-HOUGEN, HAA NIELSEN, AND A BIRCH-ANDERSEN (Copenhagen, Denmark). *Acta Pathol Microbiol Scand (C)* 1979; **87:** 217-22.

Suspensions of *Treponema pallidum* (Nichols strain) were mixed with normal and syphilitic serum and heated or unheated guinea pig serum and incubated as in the TPI test. At intervals motility counts were made and specimens examined under the electron microscope after negative staining with ammonium molybdate. After eight hours all the treponemes were immobilised in the tubes with syphilitic serum and active complement. These organisms were swollen with a distinct gap between the cytoplasmic body and the outer membrane which was obscured by fuzzy material and sometimes showed balloon-like evaginations. In contrast, treponemes incubated in medium alone, with normal serum with heated or unheated complement or with syphilitic serum and inactivated complement retained their normal morphology although up to 40% of those suspended in medium alone were non-motile. The nature of the fuzzy material is not known, but it may represent complexes of antibody with heat-labile factors in guinea pig serum.

A E Wilkinson

(Reprinted from *Abstracts on Hygiene* by permission of the Editor.)

Gonorrhoea (clinical)

Gonococcal otitis externa (Letter)
SS PAREEK (Riyadh, Saudi Arabia) *New Engl J Med* 1979; **300:** 1490.

Isolated gonococcal pulmonary valve endocarditis: diagnosis by echocardiography

BS DZINDZIO, L MEYER, R OSTERHOLM, A HOPEMAN, L WOLTJEN, AND AD FORKER (Nebraska, USA) *Circulation,* 1979; **59:** 1319-24.

Gonococcal infection in infants and children. Medical and epidemiologic considerations

YM FELMAN AND JA NIKITAS (New York, USA) *NY State J Med* 1979; **79:** 1063-5.

Gonococcal ophthalmia neonatorum after delivery by Cesarean section: report of a case

CL STRAND AND VA ARANGO (Atlanta, USA) *Sex Transm Dis* 1979; **6:** 77-8.

Superimposed traumatic and gonococcal proctitis: report of two cases

E KREUTZER AND J HANSBROUGH (Colorado, USA) *Sex Transm Dis* 1979; **6:** 75-6.

Gonorrhoea (microbiology)

New options for diagnosis and control of gonorrheal urethritis in males using uncentrifuged first voided urine (FVU) as a specimen for culture

ES MURRAY, MJF BENFARI, SR COPPOLA, MD HUGHES, WC FENG, RM MEDEIROS, AND LJ KUNZ (Boston, USA). *Am J Public Health* 1979; **69:** 596-8.

This study comprised 514 male patients with symptoms, signs, or suspicion of gonorrhoea attending a genitoinfectious diseases clinic at the Massachusetts General Hospital, Boston.

After a urethral swab had been taken for examination by culture and microscopy, the patient was asked to pass 20 ml of urine into a first voided urine (FVU) specimen collector. All FVU specimens were inoculated on to modified Thayer-Martin medium within 2-10 minutes of voiding. Culture of FVU gave positive results in 100 patients and urethral culture in 99. Seven patients, culture-positive in both types of specimen, presented with asymptomatic urethral infection. Recent urination did not affect the isolation of gonococci from either FVU or urethral specimens. FVU specimen collection was considered to be more acceptable to a male patient than a swab or loop inserted into the urethra. Unfortunately, immediate diagnosis by Gram-staining was not investigated with respect to FVU specimens.

H Young

Neisseria meningitidis and *Neisseria gonorrhoeae* bacteremia associated with C6, C7 or C8 deficiency

BH PETERSEN, TJ LEE, R SNYDERMAN, AND GF BROOKS (North Carolina and California, USA). *Ann Intern Med* 1979; **90:** 917-20.

Thirteen of 24 patients with homozygous deficiency of the sixth, seventh, or eighth components of complement, and who had had at least one episode of meningococcal or gonococcal bacteraemia, were described. It was shown that deficiency of one or other of these components was significantly associated with the development of disseminated infection and that in individuals who develop recurrent neisserial infections haemolytic complement studies are indicated.

A McMillan

Auxanographic grouping and typing of *Neisseria gonorrhoeae*

AT HENDRY AND IO STEWART (Hamilton, Canada). *Can J Microbiol* 1979; **25:** 512-21.

Release of soluble peptidoglycan from growing gonococci: hexaminidase and amidase activities

RS ROSENTHAL (Indiana, USA). *Infect Immun* 1979; **24:** 869-78.

In vivo conjugal transfer of R plasmids in *Neisseria gonorrhoeae*

M ROBERTS AND S FALKOW (Washington, USA). *Infect Immun* 1979; **24:** 982-4.

The donor strains CDC 67 and CDC 66 both produced β-lactamase mediated by a 4·4 Mdal plasmid. The former also possessed a 24·5 Mdal plasmid which has been found to promote the transfer of itself and the smaller plasmid in vitro. Both strains were sensitive to nalidixic acid and rifampin whereas the recipient strain used, F 62, was resistant to these but sensitive to penicillin. Donor and recipient strains were inoculated together into subcutaneous chambers in guinea pigs and subcultures made on to media supplemented with penicillin and nalidixic acid. The donor CDC 67, possessing the 24·5 Mdal plasmid, transferred penicillin resistance to F 62 with a frequency of 10^{-2} to 10^{-4} per recipient cell, but donor CDC 66, which lacked the large plasmid, was unable to do this. Thus R plasmid transfer was achieved in vivo in the absence of any antibiotic pressure. This strengthens the hypothesis that the 24·5 Mdal plasmid plays a part in the spread of the 4·4 Mdal plasmid from gonococcus to gonococcus in nature.

A E Wilkinson

(Reprinted from *Abstracts on Hygiene* by permission of the Editor.)

Protection by monospecific gonococcal antisera of the chicken embryo challenged with *Neisseria gonorrhoeae*

JN ROBERTSON (Southampton, England). *J Med Microbiol* 1979; **12:** 283-9.

Detection of gonococcal antigens in urine by radioimmunoassay

MJ THORNLEY, DV WILSON, RD de HORMAECHE, JK OATES, AND RRA COOMBS (Cambridge, England). *J Med Microbiol* 1979; **12:** 161-75.

A solid-phase radioimmunoassay is described which detected gonococcal antigen in urine sediments from 74% (31 of 42) of men with urethral gonorrhoea and none of 27 men with non-specific urethritis. Among women, 71% (10 of 14) with gonorrhoea were detected, but also 17% (three of 18), who did not have gonorrhoea, gave false-positive results. Compared with the Gram-stained smear, this test is less sensitive in men and less specific in women. The authors discuss possible reasons for these unimpressive results, which include the fact that the antigen used in preparation of the antiserum was derived from whole gonococci rather than surface components. It was originally intended to examine the urine supernatant until it was discovered that this contained very little antigen, the bulk being associated with pus cells in the sediment. A specificity test became necessary on each sample to correct for non-specific binding of radioactive-labelled antibody.

It is a pity that the authors did not carry out more preliminary work on their choice of antigen for raising the antiserum, and I cannot help feeling some doubts about the collection of urine samples. The importance of catching the first urine passed is not stressed in the text, which simply states "the patient's bladder contents, up to a volume of 180 ml, were collected". As it stands, the human observer aided by a simple light microscope remains more accurate and reliable than radioimmunoassay.

Brian Evans

Inhibitors in urine of radioimmunoassay for the detection of gonococcal antigens

MJ THORNLEY, MG ANDREWS, JO BRIGGS, AND BK LEIGH (Cambridge, England). *J Med Microbiol* 1979; **12:** 177-85.

It was found that the supernatant after centrifugation of 18 of 27 specimens of urine from patients with non-specific urethritis and 22 of 23 patients with gonorrhoea were inhibitory in a solid-phase radioimmunoassay system for the detection of soluble gonococcal antigens. Pus cells from urine also had an inhibitory effect. This could be abolished or reduced in most, but not in all, cases by adding soya bean trypsin inhibitor to the specimen. It is thought that inhibition is caused by proteases set free from pus cells in the urine; these act on the gonococcal antigen but not on the anti-gonococcal antibody added to the test system. Other inhibitors, not affected by the trypsin inhibitor, were also present in some specimens of urine. The centrifuged deposit from the urine of patients with gonorrhoea or non-specific urethritis also had inhibitory properties, but these could be virtually abolished by treatment with the trypsin inhibitor.

A E Wilkinson

(Reprinted from *Abstracts on Hygiene* by permission of the Editor.)

The gonococcus and the toilet seat

JH GILBAUGH AND PC FUCHS (Portland, USA). *New Engl J Med* 1979; **301:** 91-3.

Suspensions of gonococci in saline and in broth and diluted gonococcal pus were spread on a lavatory seat and on lavatory paper and cultures made at intervals. No gonococci were recovered from the areas inoculated with saline or broth suspensions after these had dried (10 minutes). Gonococci were recovered from the areas on the seat on which pus had been spread for up to two hours and from the lavatory paper for up to three hours.

No gonococci were recovered from 72 random samples from seats in public lavatories. The organisms recovered were mainly those of the skin rather than faecal flora. Although gonococci may remain viable in pus on a lavatory seat for several hours, it is difficult to visualise how they could be transferred to the genital mucosa unless indirectly by the patient's hands. Contaminated toilet paper may have a greater potential as a direct source of infection than the lavatory seat blamed by some patients.

A E Wilkinson

(Reprinted from *Abstracts on Hygiene* by permission of the Editor.)

Genetic basis for colonial variation in *Neisseria gonorrhoeae*

L NORLANDER, J DAVIES, A NORQVIST, AND S NORMARK (Ulmea, Sweden). *J Bacteriol* 1979; **138:** 762-9.

Genetic exchange mechanisms in *Neisseria gonorrhoeae*

L NORLANDER, J DAVIES, AND S NORMARK (Ulmea, Sweden). *J Bacteriol* 1979; **138:** 756-61.

Orogenital contact and the isolation of *Neisseria gonorrhoeae, Mycoplasma hominis,* and *Ureaplasma urealyticum* from the pharynx

SG SACKEL, S ALPERT, NJ FIUMARA, A DONNER, C LAUGHLIN, AND WM McCORMACK (Boston, USA). *Sex Transm Dis* 1979; **6:** 64-8.

Neisseria gonorrhoeae membrane microenvironment studied by spin-label electron spin resonance: comparison of colony types

WJ NEWHALL, FW KLEINHANS, RS ROSENTHAL, WD SAWYER, AND RA HAAK (Indiana, USA). *J Bacteriol* 1979; **139:** 98-106.

Gonorrhoea (therapy)

Prepubertal gonococcal vulvovaginitis: a penicillin-resistant infection treated with cefotoxime (Letter)

V REYNOLDS, JK OATES, AND SWB NEWSON (Cambridge, England). *Lancet* 1979; **2:** 206-7.

Comparison of ampicillin plus probenecid with amoxycillin plus probenecid for treatment of uncomplicated gonorrhea

YM FELMAN, DC WILLIAM, AND MC CORSARO (New York, USA). *Sex Transm Dis* 1979; **6:** 72-4.

Non-specific genital infection

Chlamydial infection of mothers and their infants

GT FROMMELL, R ROTHENBERG, S-P WANG, AND K McINTOSH (Colorado, Washington, and Atlanta, USA). *J Pediatr* 1979; **95:** 28-32.

Prospective screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* was performed on 340 pregnant women over a 17-month period. Infants born to 18 chlamydial culture-positive women were followed for nine months and compared with 16 chlamydia-negative controls.

Chlamydia was found in 30 (8·8%) of the 340 women, more frequently in younger, black, and unmarried women. Gonorrhoea was found in six (two chlamydial culture-positive and four culture-negative). Clinical characteristics, obstetric history, complications of labour and delivery, and evaluation of the neonate were similar in the chlamydia-positive and chlamydia-negative groups.

Eight infants from the chlamydia-positive group developed conjunctivitis (from 1 to 9 weeks of age), and seven of those had cultural evidence of chlamydial infection (in conjunctiva, nasopharynx, or rectum). Chronic pneumonitis (onset four and six weeks) was diagnosed in two of these.

One of the control culture-negative group developed conjunctivitis and recurrent wheezing diagnosed as "asthma" was found in another child after six weeks.

The nasopharynx was the most common culture-positive site (seven of the eight infants), with three rectal and two conjunctival cultures giving positive results. An overall chlamydial infection rate of 61% and evidence of clinical disease in 44% was demonstrated in the infants of chlamydial culture-positive mothers.

Comparison was made with other studies and it was suggested that: (a) intrauterine infection did not take place (as IgM concentrations in blood were not raised); and (b) postpartum transmission was possible (as breast feeding was weakly associated with the development of clinical conjunctivitis).

R S Pattman

Prospective study of chlamydial infection in neonates

J SCHACHTER, M GROSSMAN, J HOLT, R SWEET, E GOODNER, AND J MILLS (California, USA). *Lancet* 1979; **2:** 377-9.

The consequence of cervical infection with *Chlamydia trachomatis* in pregnant women was examined prospectively in their infants over a one-year period. Chlamydia was found in 4% (36/900) of pregnant women, and 20 infants born through chlamydia-infected cervices were compared with 18 infants born to chlamydia-negative mothers.

Conjunctivitis developed within 19 days in 13 neonates born to chlamydia-positive mothers; chlamydia were recovered from the conjunctivae of seven (35%) of these. Pneumonia (diagnosed by radiological examination) developed within 60-90 days in five infants exposed to chlamydia; four (20%) were associated with chlamydial infection, as judged by finding high titres of IgM class antichlamydial antibodies (geometric mean titre 1/448) in the sera. No evidence of chlamydial infection was found in those infants born through chlamydia-negative cervices (P<0·001).

A tentative estimate of infection rate was made of eight cases of chlamydial pneumonia per 1000 live births and 14 cases of chlamydial conjunctivitis per 1000 live births (total chlamydial infection rate 28 per 1000 live births).

R S Pattman

Chlamydia, cytomegalovirus, and Yersinia in inflammatory bowel disease

ET SWARBRICK, JGC KINGHAM, HL PRICE, AJ BLACKSHAW, PD GRIFFITHS, S DAROUGAR, AND NA BUCKELL (London, England). *Lancet* 1979; **2**: 11-12.

Chlamydia trachomatis in women: antibody in cervical secretions as a possible indicator of genital infection

DE McCOMB, RL NICHOLS, DZ SEMINE, JR EVRARD, S ALPERT, VA CROCKETT, B ROSNER, SH ZINNER, AND WM McCORMACK (Harvard, USA). *J Infect Dis* 1979; **139**: 628-33.

The role of local and serum antibody in the diagnosis of genital chlamydial infections is controversial. This paper attempts to clarify the predictive volume of these two parameters.

One hundred and eighty-five women college students were examined for genital infections with *Chlamydia trachomatis* and the presence of antibody in cervical secretions and serum. Of these women, 74% attended for fertility control or routine gynaecological examination and the remainder with gynaecological symptoms. One hundred and sixty (83%) were sexually experienced. *C trachomatis* was isolated from nine women, all sexually active; serum antibody was detected in these women and cervical secretion antibody in eight of the nine. Cervical antibody was detected in 14% of all the women, but none was detectable in the sexually inexperienced. Serum antibody was present in 38% of the women, including 23% of the sexually inexperienced.

The authors conclude that *C trachomatis* is sexually transmissible, with a correlation between increasing sexual experience and presence of the organism or antibody. They further suggest that cervical secretion antibody has a better predictive value for infection than serum antibody.

G L Ridgway

Activity of oral amoxycillin, ampicillin, and oxytetracycline against infection with *Chlamydia trachomatis* in mice

MJ KRAMER, R CLEELAND, AND E GRUNBERG (New Jersey, USA). *J Infect Dis* 1979; **139**: 717-9.

Susceptibility of *Chlamydia trachomatis* to antibiotics in vitro and in vivo

G JOHANNISSON, A SERNRYD, AND E LYCKE (Goteborg, Sweden). *Sex Transm Dis* 1979; **6**: 69-71.

Activity of antimicrobials against *Chlamydia trachomatis* in vitro (Letter)

GL RIDGWAY AND JD ORIEL (London, England). *J Antimicrob Chemother* 1979; **5**: 483-4.

Erythromycin and lymecycline treatment in chlamydia-positive and chlamydia-negative non-gonococcal urethritis—a partner-controlled study

A LASSUS, J PAAVONEN, M KOUSA, AND P SAIKKU (Helsinki, Finland). *Acta Derm Venereol (Stockh)* 1979; **59**: 278-81.

A group of 213 men with non-gonococcal urethritis and their sexual partners were treated either with erythromycin stearate 500 mg twice daily for 15 days or with lymecycline 300 mg twice daily for 10 or 20 days. *Chlamydia trachomatis* was isolated from 40% of the men, from 26% of their female partners, and from 56% of the partners of men with chlamydia-positive urethritis. One hundred and eighty-one men were available for evaluation of therapy. There were no signficant differences between the treatment schedules. The cure rate was 86-90% in men with chlamydia-positive urethritis and 89-100% in men with chlamydia-negative urethritis. Four of the 17 chlamydia-positive women treated with erythromycin and two of the 20 chlamydia-positive women treated with lymecycline for 10 days still harboured chlamydia on re-examination.

A McMillan

Modified metabolic inhibition test for serotyping strains of *Ureaplasma urealyticum* (T-strain mycoplasma)

JA ROBERTSON AND GW STEMKE (Alberta, Canada). *J Clin Microbiol* 1979; **9**: 673-6.

Effects of reducing agents, catalase, and reuse of medium on toxicity of media for growth on *Ureaplasma urealyticum*

IA SAYED AND GE KENNY (Washington, USA). *J Infect Dis* 1979; **139**: 720-2.

In-vitro susceptibility of *Ureaplasma urealyticum* to rosamicin

TF SMITH (Minnesota, USA). *Antimicrob Agents Chemother* 1979; **16**: 106-8.

A double-blind crossover study of the effect of doxycycline on mycoplasma infection and infertility

RA HINTON, LM EGDELL, BE ANDREWS, SKR CLARKE, AND SJ RICHMOND (Bristol, England). *Br J Obstet Gynaecol* 1979; **86**: 379-83.

The relationship of infection with *Mycoplasma hominis* and *Ureaplasma urealyticum* to infertility was examined by observing the prevalence in 42 selected infertile couples and studying the effect of a double-blind crossover trial of doxycycline and placebo.

The couples were divided into two matched groups, although a higher proportion of those who had previously never been pregnant were given doxycycline first. Monthly cervical cultures were taken for *M hominis, U urealyticum, Neisseria gonorrhoeae,* and *Candida albicans.* Monthly vaginal samples were cultured for *Trichomonas vaginalis* and an initial cervical sample cultured for *Chlamydia trachomatis.* Semen samples were cultured for *M hominis* and *U urealyticum* and three postcoital tests were performed during the trial. Doxycycline was administered in three 10-day courses, to one group in relation to the menstrual cycle (over three months) and placebo was administered in the same way to the second group. After three months those who had taken doxycycline were given placebo and those previously taking placebo were given doxycycline.

Infection with *U urealyticum* and *M hominis* was found in 21 couples and with *C albicans* in two women; no other pathogens were isolated. Of the 21 couples infected, 19 were cured with doxycycline; of these, four women became pregnant. Pregnancy was achieved by one couple free from infection, one couple before treatment started, and one after the trial was over.

As pregnancy occurred within the first four months of the trial in five of the seven women, it was suggested that the psychological effect of taking treatment was of more importance than the effect of the drug on the infection.

R S Pattman

Etiology, manifestations and therapy of acute epididymitis: prospective study of 50 cases.

RE BERGER, ER ALEXANDER, JP HARNISCH, CA PAULSEN, GD MONDA, AND KK HOLMES (Washington, USA). *J Urol* 1979; **121:** 750-4.

Between 1972 and 1977, at Seattle, a prospective study was carried out of 50 men presenting with acute epididymitis and who had received no antibiotic treatment within the preceding month.

Swabs were taken from the urethra for culture of *Neisseria gonorrhoeae* and *Chlamydia trachomatis.* Mid-stream urine specimens for culture of aerobic bacterial pathogens were taken from all 16 patients aged 35 years and over and from 22 of 34 men under the age of 35. Cultures for *Ureaplasma urealyticum* and *Mycoplasma hominis* were made on first-voided urine specimens or on urethral specimens from 16 men over the age of 35 and from 28 younger patients. In 11 of the latter patients, expressed prostatic secretion was also cultured for aerobic and anaerobic pathogens, *N gonorrhoeae, U urealyticum, M hominis,* and *C trachomatis.* Similar microbiological studies were made on 19 epididymal aspirates. Serum was taken on the first visit and again two to six weeks later and was tested for antibody to *C trachomatis* by microimmunofluorescence. Eight sexual partners were investigated, and culture specimens collected for the above organisms. Treatment depended on which organisms were isolated and followed standard practice.

Escherichia coli was isolated from 12 of 16 men aged 35 and over but from only one of 28 younger patients. *N gonorrhoeae* and *C trachomatis* were isolated in 23 of 34 men under the age of 35 but only one of 16 older men ($P<0 \cdot 0005$). Of all patients under 35 who did not have gonococcal or Gram-negative rod infections or who had not suffered direct trauma to the scrotal contents, 16 (67%) of 24 had a positive culture result for *C trachomatis.* Serum antibody to *C trachomatis* was detected in 16 of 17 men from whom the organism was isolated and in 11 of 29 who had negative cultures ($P<0 \cdot 0002$). Epididymal aspirates confirmed the findings of the urethral culture. *U urealyticum* was isolated from eight of 10 men with no other apparent aetiological agent, from two of five men with gonorrhoea, and from nine of 16 with chlamydial infection. No new observations were made on the well recognised features of epididymitis. All patients with gonococcal or coliform epididymitis responded to specific therapy. Response was also satisfactory in 20 patients with nongonococcal, non-coliform epididymitis treated with tetracycline for 10 days.

E coli was the predominant pathogen isolated from the urine of men over the age of 35 whereas *C trachomatis* and *N gonorrhoeae* were the predominant pathogens found in younger men. Chlamydial epididymitis accounted for two-thirds of idiopathic epididymitis in young men and was associated with oligospermia. In the sexual partners of men with chlamydial infection, six had serum antibody to *C trachomatis,* two had positive chlamydial culture results from the cervix, and another two had nongonococcal pelvic inflammatory disease. It was concluded that tetracycline should be offered to female sexual partners of men with epididymitis.

M Waugh

Trichomoniasis

Metronidazole and the fetal alcohol syndrome (Letter)

PM DUNN, S STEWART-BROWN, AND R PEEL (Bristol, England). *Lancet* 1979; **2:** 144.

Mutagenicity of metronidazole (Letter)

B HARTLEY-ASP (Helsingborg, Sweden). *Lancet* 1979; **1:** 275.

Metronidazole is mutagenic in bacteria, and a possible chromosome-breaking activity has been reported during the treatment of patients with Crohn's disease.[1]

Twelve women with trichomoniasis were treated with 200 mg metronidazole three times daily for seven days. Blood was collected before treatment, on the seventh day, and three weeks after the end of treatment, and cultures were set up for chromosome analysis; samples of 100 cells per patient were analysed for chromosomal aberrations. No chromosome-breaking activity was detected and metronidazole is thought to be a safe drug for short-term treatment.

A E Wilkinson

(Reprinted from *Abstracts on Hygiene* by permission of the Editor.)

Reference

1. Mitelman F, Hartley-Asp B, Ursing B. *Lancet* 1976; **1:** 802.

Candidosis

Lactobacillus overgrowth for treatment of moniliary vulvovaginitis (Letter)

TE WILLS (Minnesota, USA). *Lancet* 1979; **2:** 482.

Isolation and characterization of a polyene-resistant variant of *Candida tropicalis*

WG MERZ AND GR SANDFORD (Baltimore, USA). *J Clin Microbiol* 1979; **9:** 677-80.

Autoregulation of germ tube formation by *Candida albicans*

KC HAZEN AND JE CUTLER (Montana, USA). *Infect Immun* 1979; **24:** 661-6.

Detection of *Candida* antigen in sera of patients with candidiasis by an enzyme-linked immunosorbent assay-inhibition technique

E SEGAL, RA BERG, PA PIZZO, AND JE BENNET (Maryland, USA). *J Clin Microbiol* 1979; **10:** 116-8.

Genital herpes

Immunotherapy and drugs in neonatal disseminated herpes simplex virus type 2 infections: a mouse model

C STRULOVITCH, MI MARKS, A SONEJI, AND S GOLDBERG (Montreal, Canada). *J Antimicrob Chemother* 1979; **5:** 437-46.

Inhibition of herpes virus by a new compound—acyclic guanosine

JS OXFORD (London, England). *J Antimicrob Chemother* 1979; **5:** 333-4.

Herpetic proctitis and sacral radiomyelopathy—a hazard for homosexual men

PL SAMARSINGHE, JK OATES, AND IPB MacLENNAN (London, England). *Br Med J* 1979; **2:** 356-6.

Colposcopy in a case of suspected genital herpes infection in early pregnancy
JM van MEIR, AC DROGENDIJK, JW WLADMIROFF, AND G WIELENGA (Rotterdam, the Netherlands). *Acta Obstet Gynecol Scand* 197[illegible]; **58:** 221-3.

Other sexually transmitted diseases

Comparison of two media for isolation of *Haemophilus vaginalis*
RF SMITH (California, USA). *J Clin Microbiol* 1979; **9:** 729-30.

A reservoir of hepatitis B
KS LIM, RD CATTERALL, R SIMON, DS DANE, M BRIGGS, AND RS TEDDER (London, England). *J Infect* 1979; **1:** 163-70.

A total of 7300 patients attending a clinic for sexually transmitted diseases were screened for hepatitis B surface antigen (HBsAg). The 72 HBsAg-positive sera were tested for e antigen and antibody (HBe and anti-HBe) by immunodiffusion and radioimmunoassay and the patients were followed up to find out whether they were HBsAg-carriers or suffering from acute hepatitis B infections. Most of the HBe-positive patients were found to be carriers. Seventeen of 19 HBe-positive carriers and 14 of 16 patients with acute infections were male homosexuals. Fourteen (58%) of 24 male homosexual carriers from the United Kingdom or other countries with a low prevalence of hepatitis B were HBe-positive. The effect of identifying and then treating or counselling HBe-positive HBsAg carriers is discussed.

Authors' summary

Homosexual hepatitis
JC COLEMAN, BA EVANS, A THORNTON, AND AJ ZUCKERMAN (London, England). *J Infect* 1979; **1:** 61-6.

Public health and social aspects

Gonorrhea in street prostitutes: epidemiologic and legal implications
JL POTTERAT, R ROTHENBERG, AND DC BROSS (Colorado, USA). *Sex Transm Dis* 1979; **6:** 58-63.

Foreign Office survey of venereal disease and prostitution control 1869-1870
JB POST. *Med Hist* 1978; **22:** 327-34.

Miscellaneous

Sexually transmitted enteric diseases
YM FELMAN AND NB RICCIARDI (New York, USA). *Bull NY Acad Med* 1979; **55:** 533-9.

Contact reaction of penicillamine in vaginal secretions (Letter)
PCH NEWBOLD (Worcester, England). *Lancet* 1979; **1:** 1344.

Genital actinomycosis (Letter)
EM TIAMSON (Baltimore, USA). *Hum Pathol* 1979; **10:** 119-20.

The association of histocompatability antigens of the B7 cross-reacting group with Peyronie's disease
MK WILLSCHER, WF CWAKA, AND DE NOVICKI (California, USA). *J Urol* 1979; **122:** 34-5.

FORTY-EIGHT HOUR PATIENCE

One tablet nightly for three nights means that your patient's patience only needs to last 48 hours. That's all it takes to clear vaginal candidiasis – twice as conveniently as the old One Two Free.

Canesten®
clotrimazole

New 200mg vaginal tablet now only one a night for three nights

Presentations Canesten is available either as 200 mg vaginal tablets in packs of three with applicator or as 100 mg vaginal tablets in packs of six with applicator. Canesten Duopak contains six 100 mg vaginal tablets with applicator plus 20 g cream containing 1.0% clotrimazole. **Dosage** *Vaginal Tablets 100mg* Two vaginal tablets should be inserted at night for three consecutive days. Alternatively, one vaginal tablet daily for six days. *Vaginal Tablets 200mg* One vaginal tablet should be inserted at night for three consecutive days. *Duopak* Vaginal Tablets 100 mg as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to prevent re-infection. **Side effects** Rarely patients may experience transient local mild burning or irritation. **Basic NHS Costs** 6 x 100 mg vaginal tablets with applicator £2.13; 3 x 200 mg vaginal tablets with applicator £1.99; Duopak £3.47. **Product Licence Numbers** Vaginal tablets 100 mg, PL 0010/0015; vaginal tablets 200 mg, PL 0010/0072; Duopak, PL 0010/0016 & PL 0010/0015. Further information is available from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. ® Registered Trade Mark of Bayer Germany.

THEY CALL IT CRABS
You may know it as Phthirus pubis

Whatever you call them, pubic lice are a problem, because any measure you adopt to eradicate them must take care of both adult lice and nits, while being cosmetically acceptable. Quellada Shampoo fills the bill particularly well. Its active constituent, gamma benzene hexachloride, is rapidly lethal to crab lice, while the foaming shampoo base ensures that Quellada penetrates through to the nits. So a single application of Quellada kills lice *and* nits. Quellada Shampoo is easy and pleasant to use, because it doesn't smell or irritate the skin.

Quellada PC Shampoo
gamma benzene hexachloride 1%
kills pubic lice and nits in 4 minutes.

Presentation
Gamma benzene hexachloride 1% in a specially formulated foaming shampoo base.

Uses
Antiparasitic and ovacidal. For use on hairy areas of the body in the treatment of lice infestations, e.g. head lice, body lice, pubic lice.

Dosage and administration
Apply to wet hair, rub vigorously for four minutes, rinse and comb with fine-tooth comb.

Contra-indications, Warnings etc.
Avoid contact with the eyes. Should lather accidentally get into eyes, flush immediately with water. Do not use more than twice in any one infestation nor in any one week. Do not use on broken or infected skin.

Packaging and price
Bottles of 100 and 500ml. Basic NHS cost 60p for 100ml.

Product Licence No.
0036/5001.

Further information is available on request.

Stafford-Miller Limited,
Professional Relations Division,
Hatfield, Herts. AL10 0NZ.

Sexually Transmitted Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $25.00 outside the United States ($22.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $28.00 in the United States and $30.00 in all other countries.

Michael F. Rein, M.D.
American Venereal Disease Association
Box 385
University of Virginia School of Medicine
Charlottesville, Virginia 22901 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $25.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases*.
☐ I wish to enter a Non-Member or institutional subscription ($28.00 U.S., all other countries $30.00).
(Mail non-member subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105)

Name ______________________________

____

Address ______________________________

City ______________________ State Country ______________ Zip ____________

British Journal of Venereal Diseases February 1980 Vol. 56 No. 1

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

The International Union against the Venereal Diseases and Treponematoses
R D CATTERALL (Gt Britain) *page* 1

Characterisation of *Neisseria gonorrhoeae* from women with simultaneous infections at two sites
ROBERT C NOBLE (USA) *page* 3

Homosexuality and venereal disease in the United Kingdom
BRITISH CO-OPERATIVE CLINICAL GROUP (Gt Britain) *page* 6

Recent observations on the serology of syphilis
A LUGER, B SCHMIDT, I SPENDLINGWIMMER, AND F HORN (Austria) *page* 12

Non-specific positive test results to syphilis in dermatological diseases
M GIBOWSKI AND E NEUMANN (Poland) *page* 17

Antitreponemal IgE in early syphilis
J D BOS, F HAMERLINCK, AND R H CORMANE (the Netherlands) *page* 20

Auxotypes and antimicrobial suceptibilities of *Neisseria gonorrhoeae* in black and white patients
ROBERT C NOBLE AND B R MILLER (USA) *page* 26

Relationship of the interval between infections and the similarity of gonococcal strains in recurrent gonorrhoea
ROBERT C NOBLE, A P ORANJE, M F MICHEL, AND E STOLZ (USA and the Netherlands) *page* 31

An unusual case of gonococcal arthritis
K M RENNEY (USA) *page* 35

Chlamydial infection of the cervix in contacts of men with nongonococcal urethritis
I A TAIT, E REES, D HOBSON, R E BYNG, AND M C K TWEEDIE (Gt Britain) *page* 37

Urine culture for the detection of *Trichomonas vaginalis* in men
A WILSON AND J P ACKERS (Gt Britain) *page* 46

Herpesvirus hominis type 2 infection in Ibadan
D MONTEFIORE, A O SOGBETUN, AND C N ANONG (Nigeria) *page* 49

Sexually transmitted diseases in clinic patients in Lagos
V O ROTIMI AND A O SOMORIN (Nigeria) *page* 54

Correspondence *page* 57

Notices *page* 57

Book review *page* 58

Abstracts *page* 59

ASTM CODEN: BJVDAK 56 (1) 1-64 (1980)

ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

22.10.80

April 1980 Vol. 56 No. 2

British Journal of
Venereal Diseases

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

R N THIN *(Editor)*
J D ORIEL
B EVANS
A McMILLAN *(Abstracts)*
(Assistant Editors)
C B S SCHOFIELD
(President, MSSVD)

J BARROW
(Hon. Sec., MSSVD)
J WALLIN
G CAUSSE
J M KNOX
A E WILKINSON

I PHILLIPS
G CSONKA
E STOLZ
J THIVOLET
L M DRUSIN

EDITOR
British Medical Journal
SHUNA JUDGE
(Technical Editor)

This *Journal,* founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A E Wilkinson, 22 Kingsend, Ruislip, Middlesex HA4 7DA. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals,* available from the Publishing Manager, *British Medical Journal,* BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; **55**: 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS. *Venereal Diseases.* 3rd ed. London: Baillière and Tindall, 1975.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 214-22.

PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £23.00 in the United Kingdom and Republic of Ireland and US$55.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US$55.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal,* 1172 Commonwealth Avenue, Boston, Mass. 02134. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases,* BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1980 by the *British Journal of Venereal Diseases.* This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.

Ecostatin™

* econazole nitrate

"Once the infection has been established in the female, it can undoubtedly be transmitted to the male consort... This may, in turn... serve as a reservoir for the reinfection of his partner after her condition had responded successfully to treatment. The risk of this sexual 'ping-pong' has been recognised..."[1]

the most active fungicide available prevents 'sexual ping-pong'

ECOSTATIN*
Pessaries: Each contain 150 mg econazole nitrate.
Twin Pack: Three pessaries plus 15 g Ecostatin cream, 1% w/w econazole nitrate.

Indications
Pessaries and Twin Pack: Vulvovaginal candidosis.

Actions
Econazole nitrate is a broad-spectrum antifungal agent.

Dosage and Administration
Pessaries and Twin Pack: One pessary to be inserted at bedtime for three consecutive nights. The cream to be applied twice daily to the anogenital area.

Precautions
The pessaries should not be used during the first trimester of pregnancy unless deemed essential for the patient's wellbeing. Topical preparations should not be used in or near the eyes and used with care in the presence of eczematous dermatitis.

Side-effects
Occasional local irritation. The pessaries may cause transitory discomfort.

Package quantities, prices and Product Licence Numbers
Pessaries: Pack of 3 with applicator - £3.60
PL No. 0085/0013
Twin Pack: 3 pessaries, applicator and 15 g cream - £4.70

Cream: 15 g - £1.25;
30 g - £2.50
PL No. 0085/0014

Product Licence Holder
F.A.I.R. Laboratories Ltd.,
179 Heath Road,
Twickenham, TW1 4BJ

(1) Br. J. Sex. Med. 1974, 1, 21.

Just one pessary a night for three nights clears candidal vaginitis. The cream treats the anogenital region, and its simultaneo[illegible] male partner will prevent re-infection.

- **[illegible] pessary disintegr[illegible] independent of vaginal secretion.**
- **[illegible] pessaries melt and disperse within ten minutes [illegible] al wall.**
- **[illegible] nd acceptable presentation [illegible] proved p[illegible] compliance.**
- **[illegible] sistance has n[illegible] been observed.**
- **[illegible] e-infection and can be used to [illegible] ual p[illegible] er.**

Further information is available from
FAIR Laboratories Ltd., 179 Heath Road, Twickenham, Middx. TW1 4BJ
**Ecostatin is a registered trademark and FAIR Laboratories is the authorised user*

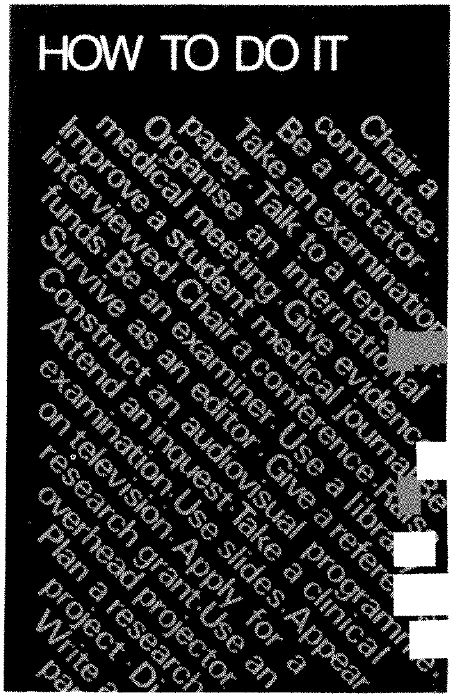
FAIR

HOW TO DO IT

Every doctor knows the sinking sensation induced by waiting one's turn for an examination viva and the social anxieties of changing jobs. The misery of first night nerves does not, however, disappear with time: each decade brings new challenges. On these occasions—chairing a committee, giving evidence in court, appearing on television—practical tips can be enormously helpful. The same is true of the many skills not taught in medical school—how to lecture, referee papers, edit, or use a library. All these and many more nuggets of down-to-earth advice have been collected from past issues of the *BMJ* to form a compendium for the novice—whatever his or her age or seniority.

Price: Inland £4.00;
Abroad US$10.00
(Concessionary price to BMA members: Inland £3.50;
Abroad US$8.75.
When ordering BMA members must quote their membership number or the full price will be applicable.)
Payment must be enclosed with order or a surcharge of 30p will be made for rendering invoices and statements.

Order your copy now
From: The Publisher
British Medical Journal
BMA House
Tavistock Square
London WC1H 9JR
or any leading bookseller


HOW TO DO IT
Chair a committee. Be a dictator. Take an examination. paper. Talk to a repo Organise an international medical meeting. Give evidenc Improve a student medical journal. Be interviewed. Chair a conference. Raise funds. Be an examiner. Use a libra Survive as an editor. Give a refere Construct an audiovisual programme. Attend an inquest. Take a clinical examination. Use slides. Appear on television. Apply for a research grant. Use an overhead projector. Plan a research project. D Write a pa

THEY CALL IT CRABS

You may know it as Phthirus pubis

Whatever you call them, pubic lice are a problem, because any measure you adopt to eradicate them must take care of both adult lice and nits, while being cosmetically acceptable. Quellada Shampoo fills the bill particularly well. Its active constituent, gamma benzene hexachloride, is rapidly lethal to crab lice, while the foaming shampoo base ensures that Quellada penetrates through to the nits. So a single application of Quellada kills lice *and* nits. Quellada Shampoo is easy and pleasant to use, because it doesn't smell or irritate the skin.

Quellada PC Shampoo

gamma benzene hexachloride 1%

kills pubic lice and nits in 4 minutes.

Presentation
Gamma benzene hexachloride 1% in a specially formulated foaming shampoo base.

Uses
Antiparasitic and ovacidal. For use on hairy areas of the body in the treatment of lice infestations, e.g. head lice, body lice, pubic lice.

Dosage and administration
Apply to wet hair, rub vigorously for four minutes, rinse and comb with fine-tooth comb.

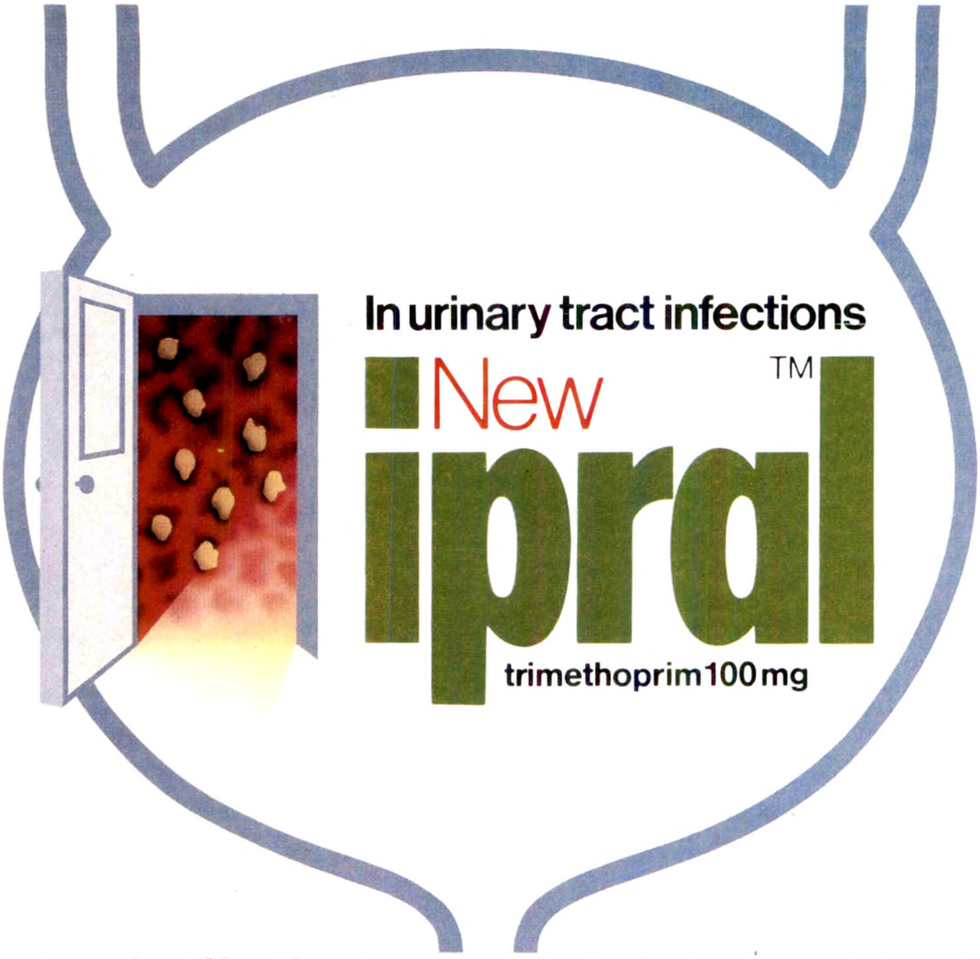
Contra-indications, Warnings etc.
Avoid contact with the eyes. Should lather accidentally get into eyes, flush immediately with water. Do not use more than twice in any one infestation nor in any one week. Do not use on broken or infected skin.

Packaging and price
Bottles of 100 and 500ml. Basic NHS cost 60p for 100ml.

Product Licence No.
0036/5001.

Further information is available on request.

Stafford-Miller Limited,
Professional Relations Division,
Hatfield, Herts. AL10 0NZ.



In urinary tract infections

New ipral™

trimethoprim 100 mg

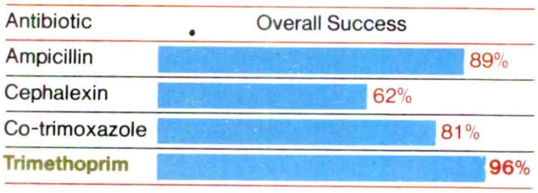
...the way to effective treatment of urinary tract infections

Trimethoprim – proved effective as a single entity without the side-effects associated with sulphonamides.

"Trimethoprim alone may well prove as effective as trimethoprim-sulphamethoxazole combinations ..."[1]

96% success in general practice.[2] Half as many side-effects.

Results of 107 Courses of Treatment in 96 Domiciliary Patients



ipral simple treatment for acute infections – 2 tablets b.d.

ipral for routine use in chronic U.T.I. – 1 tablet b.d.

In a study on recurrent U.T.I.'s
"... trimethoprim is the most effective of the antibacterial agents examined."[3]

Greater efficacy with fewer side effects.

In the trial in acute U.T.I.'s

"... the total side effects caused by ampicillin, cephalexin and co-trimoxazole were similar and occurred twice as often as when trimethoprim was given alone ..."[2]

Antibiotic	No. of Patients	% Side Effects
Ampicillin	88	22
Cephalexin	84	25
Co-trimoxazole	83	21
Trimethoprim	**84**	**8**
Total	339	

Prescribing information

IPRAL tablets
Each tablet contains trimethoprim B.P. 100 mg

Indications
Ipral is indicated for the treatment and prophylaxis of acute and chronic urinary tract infections.

Actions
Ipral is active in-vitro against most pathogenic Gram-positive and Gram-negative bacteria. Exceptions include Neisseria species and Pseudomonas aeruginosa.

Dosage and administration
For acute urinary tract infections 2 tablets twice daily is recommended. For long term and prophylactic therapy 1 tablet twice daily is recommended.

Contra-indications
Ipral should not be given to patients with severe renal insufficiency where blood levels cannot be monitored regularly. Ipral should not be used in pregnancy or during the first few weeks of life.

Precautions
Care is necessary in administration to patients with impaired renal function. Special precaution should be exercised in patients with a predisposition to folate deficiency.

Side-effects
Nausea, vomiting and skin rashes may occur but are rare. Ipral may depress haematopoiesis during prolonged therapy. This has proved reversible when treated with calcium folinate.

Treatment of overdosage
Gastric lavage. Calcium folinate may be used to counteract any effect of Ipral on bone-marrow.

Package quantities and price
Packs of 100 tablets £5.30.
Product Licence No. – 0034/0190

Full prescribing information available from:
Technical Services Department, E. R. Squibb & Sons Ltd
Regal House, Twickenham, TW1 3QT
Tel: 01-892 0164

5280 Oct 79

Ipral is a Trade Mark of E. R. Squibb & Sons Ltd

...the way to effective treatment of urinary tract infections

- As effective as trimethoprim/sulphamethoxazole combination
- Avoids the side effects of sulphonamides
- At a sensible price

and "Trimethoprim given alone was shown to be much better tolerated."[2]

"Treatment with TMP (trimethoprim) alone is superior to the combined regimen due to a smaller incidence of side-effects; ..." thus "... introduction of TMP alone on the market is recommended."[4]

"Less gastrointestinal side-effects and skin rashes have occurred with trimethoprim ..."[3]

References
1. Lancet 1979, **1**, 493. 2. Br. med. J. 1972, **2**, 673.
3. Ann. clin. Res. 1978, **10**, (suppl. 22). 4. Chemother. 1973, **19**, 314.

New ipral™
trimethoprim 100 mg

more than half the story

SQUIBB™ Full prescribing information available from Technical Services Department, E. R. Squibb & Sons Ltd. Regal House, Twickenham, TW1 3QT. Tel. 01-892 0164

UNIVERSITY OF LIVERPOOL

Diploma in Venereology

The annual full-time three-months course in venereal and sexually transmitted diseases, leading to the Diploma in Venereology, of the University of Liverpool, will commence in January 1981.

The course covers the whole field of sexually transmitted infection and includes lectures and laboratory instructions.

Application forms are available from:

Department of Venereology,
'Q' Clinic,
Royal Liverpool Hospital,
Prescot Street,
LIVERPOOL L7 8XP.

and should be returned, duly completed by 15th May, 1980.

Drugs and Disease

The Proceedings of a Symposium
organized by the
Royal College of Pathologists

Edited by
Sheila Worlledge

Price: Inland £3·00
Abroad US$7·50
including postage

The Publishing Manager, JOURNAL OF
CLINICAL PATHOLOGY, BMA House,
Tavistock Square, London WC1H 9JR

TODAY'S TREATMENT/3

Diseases of the respiratory and urinary systems account for a high proportion of all consultations in general practice, justifying the 200 pages devoted to them in the third volume of Today's Treatment. The remaining section deals with the use of antibiotics across the whole spectrum of medicine, looking at the advantages and drawbacks of the major categories and their use in clinical settings where decision-making may be difficult. Like its predecessors, Today's Treatment/3 concentrates on practical issues and provides the busy clinician with the information he needs in his day to day practice.

Demy Octavo—Paperback—318 pages—ISBN 0 7279 0036 6

Price: Inland £5·50; Abroad US$13.75, including postage

(Concessionary price to BMA Members: Inland £5·00; Abroad US$12.50. When ordering BMA Members must quote their membership number or the full price will be applicable.)

Payment must be enclosed with order or a surcharge of 50p will be made for rendering invoices and statements.

Order your copy now from
The Publisher, BRITISH MEDICAL JOURNAL, BMA House,
Tavistock Square, London WC1H 9JR *or through any leading bookseller*

IMPORTANT NEW BOOK

TROPICAL VENEREOLOGY

O. P. Arya, A. O. Osoba and *F. J. Bennett*

1980 248 pages 48 half-tone + 5 line illustrations paperback £8.00

The majority of books on venereology are based on data obtained from, and methods employed in, the developed countries of the world. This book recognises the differences experienced when working in tropical areas. The authors, while indicating ideal courses of action, also provide guidelines to a more pragmatic approach. They believe that such rationalisation will bring help to the greatest number of patients, within the limited means and facilities usually available.

Sexually Transmitted Diseases

C. B. S. Schofield

1979 Third edition 288 pages illustrated paperback £3.75

'Students will find this edition comprehensive and helpful in their study and understanding of these diseases'.

Nursing Times

In preparation

Recent Advances in Sexually Transmitted Diseases—2

Edited by *J. R. W. Harris*

Contributions from experts in centres in the USA, Europe, India and Africa.

Scheduled for September publication.

Churchill Livingstone

Robert Stevenson House, 1-3 Baxter's Place, Leith Walk, Edinburgh EH1 3AF

British Postgraduate Medical Federation
University of London

Postgraduate Course in Sexually Transmitted Diseases

A twelve week full-time course in SEXUALLY TRANSMITTED AND RELATED DISEASES is held twice a year, starting in September. This is a comprehensive course, intended for those with a particular interest and some experience in the specialty. For clinical teaching, postgraduates are attached to the departments of genito-urinary medicine/venereology in the number of London teaching hospitals. The whole course meets together once a week (usually on Fridays) for an academic Seminar Day, of lectures, discussions, case demonstrations etc.

The programme is so arranged that a limited number of doctors already working in the specialty in this country, may attend the Seminar Days only on a part-time, day release basis.

The number accepted on each course is limited, and early application is recommended, for both full and part-time attendance.

Future courses: September 22 to December 12 1980. March 23 to June 12 and September 21 to December 11 1981. Fees: £200 full-time, £80 part-time.

Application forms and further details from the Secretary to the STD Course, British Postgraduate Medical Federation,
33 Millman Street, London WC1N 3EJ.

MINOCIN

Minocycline
Hydrochloride
100 mg

TABLETS

for the treatment of gonorrhoea and non-specific urethritis[1-4]

1 Brit. J. Vener. Dis. (1975), 51, 97–98
2 Brit. J. Vener. Dis. (1975), 51, 206–209
3 Brit. J. Vener. Dis. (1976), 52, 43–45
4 Brit. J. Vener. Dis. (1978), 54, 107–111

INFORMATION ON THE USE OF MINOCIN IN THE TREATMENT OF VENEREAL DISEASE AND PRODUCT DATA WILL BE SUPPLIED UPON REQUEST

Lederle Laboratories
Research for British Medicine
A division of Cyanamid of Great Britain Ltd
Fareham Road, Gosport, Hants PO13 0AS

MINOCIN is a trademark for minocycline hydrochloride

Br J Vener Dis 1980;**56**:65-8.

Some aspects of venereal diseases in Greenland

ELLIS FROM

From the Department of Dermatology and Venereology, University of Aarhus, Marselisborg Hospital, Aarhus, Denmark

SUMMARY For many years venereal diseases have been a major problem in Greenland. Since the early 1950s gonorrhoea has been widely prevalent; in the middle of the 1960s the incidence increased rapidly. Since the early 1970s syphilis has become widespread in the population, and in 1977 an epidemic of chancroid occurred. The high figures for venereal diseases are basically due to the social change towards a modern industrial society. Improvements in the health services could be made by encouraging health education on venereal diseases, training Greenlander health personnel, and appointing a venereologist.

Introduction

Greenland is the world's largest island with a surface area of just under 2·2 million km^2, about 80% of which is covered by the ice cap. The distance from north to south is about 2700 km—that is, as far as from southern Norway to Tunisia. The distance from east to west is 1050 km, or as far as from Copenhagen to Paris. The Greenlanders have a language of their own; many young Greenlanders also speak Danish.

The population is almost 50 000, of whom nearly 10 000 are from Denmark. Before the second world war the inhabitants were distributed equally between the towns and settlements, but during recent years the population has concentrated in the towns, so that about 75% now live there.

In previous years, the population lived on hunting —especially seal hunting—and the standard of living was determined mainly by the efforts of the individual hunter. On the amendment of the Danish constitution in 1953, after which Greenland acquired the status of part of the Kingdom of Denmark with equal rights, special importance was attached to fishing. By Danish capital transfers, efforts have been made to change Greenland into a modern industrial society with social welfare benefits. This has resulted in a considerable improvement in the standard of living and an explosive increase in the population, which has now been curbed by intensive contraceptive programmes.

Greenland has a free health service, including free hospital treatment and free medicines. Greenland is divided into 16 medical districts, each served by up to five doctors. In each district there are nurses, mainly from Denmark, and a native nursing staff, all of whom have received a three-year hospital training in Greenland.

In the spring and summer of 1977, I went to Greenland to evaluate the prevalence of venereal diseases there. I visited 14 of the 16 medical districts; the remaining two districts were remote hunters' districts, in which venereal disease was not a problem. The population over 13 years of age in these two districts consisted of 442 men and 416 women—that is, 2·3 and 2·6% respectively of the entire male and female population over 13 years.[1]

Gonorrhoea

For many years the venereal diseases have been a major problem in Greenland and the fight against them has put a considerable strain on the health services. It was more than 140 years since the Danish colonisation of Greenland in 1721 before venereal disease in Greenlanders was first observed. The first case was one of gonorrhoea among women living in the neighbourhood of Ivigtut, the cryolite mine in southern Greenland.[2] During the next 50 years only a few cases were reported. From 1913 to 1940, the prevalence of gonorrhoea varied greatly from year to year. A remarkably high percentage of infected patients (68%) were married and 35% were over 30 years of age. Nearly as many women were affected as men; of the latter, many were both elderly and married.[3]

Address for reprints: Dr E From, Department of Dermatology and Venereology, Marselisborg Hospital, DK 8000 Aarhus C, Denmark

Received for publication 17 September 1979

Since 1945 the prevalence of gonorrhoea has been higher in Greenland than in Denmark. From 1966 to 1974 (when nearly 12 000 cases were reported) the incidence rose steeply (Fig 1). The increase has been most pronounced in the 16-19 age range, particularly among girls. The large number of repeated infections is a characteristic feature of the gonorrhoea morbidity in Greenland. Of the teenagers, 57% are reinfected within one year; 31% of girls and 11% of boys are treated more than twice a year for gonorrhoea.[4] During the last few years the number of reported cases has fallen. During the first nine months of 1977, a total of 7608 cases were reported in the 14 medical districts that I visited—4533 in men and 3075 in women. The distribution per 1000 population by age and sex is given in Table I. The high prevalence in young women is remarkable. Furthermore, gonorrhoea was diagnosed in three boys and nine girls aged from 7 to 12 years. In the other two medical districts, there were 35 cases of gonorrhoea in men and 40 cases in women.

Syphilis

Until 10 years ago, Greenland was largely free of syphilis. Small localised outbreaks had occurred in 1872, 1947, and 1965 in southern Greenland.[5 6] That Greenland could previously be kept free of syphilis was undoubtedly due to its only connection with the outside world being by ship from Denmark. Medical examination of all crews was compulsory before departure to Greenland. The voyage was so long that the incubation period for syphilis was usually exceeded before the ships arrived and any signs of syphilis on arrival led to quarantine of the person concerned.

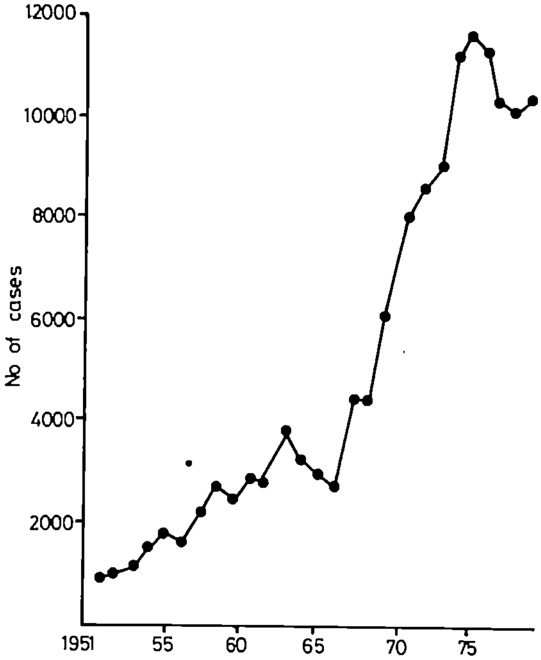


FIG 1 *Annual number of reported cases of gonorrhoea in Greenland*

TABLE I *Age and sex distribution of number of reported cases of gonorrhoea, syphilis, and chancroid per 1000 population (1 January to 1 October 1977)*

	Age group (years)							
	13-15		*16-19*		*20-44*		*45 or over*	
Disease	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
Gonorrhoea	21	93	386	565	352	217	42	21
Syphilis	1	12	17	48	22	20	6	5
Chancroid	0·5	7	36	35	28	17	6	3

However, after 1969 an increasing number of cases occurred, and during the following few years this increase was exponential (Fig 2). During the first nine months of 1977 a total of 577 cases—284 in men and 293 in women—were reported in the 14 medical districts visited. The sex and age distribution per 1000 population is shown in Table I. Again, the high prevalence among girls aged 13-19 years is remarkable. In addition, one case of congenital syphilis was detected in a newborn baby and two cases of acquired syphilis were diagnosed in boys aged 8 and 9 years. In the other two medical districts, there were two cases of syphilis.

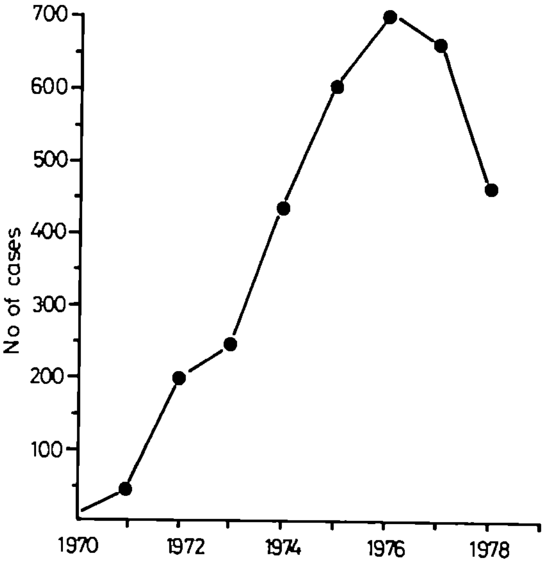


FIG 2 *Annual number of reported cases of syphilis in Greenland*

In 1977 the increase in the incidence of syphilis suddenly stopped and a decline occurred (Fig 2). Several factors have presumably contributed to this decline. One important factor was the epidemic of chancroid, which alarmed the population. The painful genital ulcers caused many patients to seek medical advice early, which led to a more prompt diagnosis of syphilis. Another important factor is that during recent years epidemiological treatment has consistently been given to all contacts of syphilis, thus causing a decrease in the pool of infectious syphilis from which the infection is contracted.

Within the last two years the rapid plasma reagin test has been widely used in Greenland, thus making it possible to establish the diagnosis at an earlier stage and to shorten the infectious period. Previously, the diagnosis depended on the result of the Wassermann reaction—which is performed at Statens Serum Institute in Copenhagen—and, when weather was bad, two to three weeks could pass before treatment could be started, which obviously prolonged the infectious period. New darkground equipment in the various medical districts has also contributed to an earlier diagnosis and a shortening of the infectious period.

So far, no cases of tertiary syphilis have been encountered. However, many hidden cases of syphilis do exist. Screening of the adult population, using the Wassermann reaction, carried out in three towns during the spring of 1977 showed that nearly 1% of adults had previously had unrecognised syphilis. Patients of all different age and socioeconomic groups, as well as of marital state, were affected. This confirms the old venereological truth that syphilis spreads diffusely and secretly among the whole population. However, the figure of 1% is low compared with the very high figures shown by screening programmes in some settlements in southern Greenland in 1976; about 40 cases were detected, although only 300 persons were investigated.

Chancroid

We do not know when chancroid came to Greenland, but presumably it was in 1976. For some years, Greenland has had rapid communications with the outside world, both by ship and by air. In 1976 patients with painful genital ulcers—in some cases associated with abcesses in the groin—were found in some towns on the west coast. Gradually it became clear that the patients had chancroid. The epidemic spread rapidly in Godthåb in March 1977 and in the following months in southern Greenland (Fig 3). In the summer months a few cases occurred in the Disko area. No cases occurred in northern or eastern Greenland, presumably because of the longer travelling time.

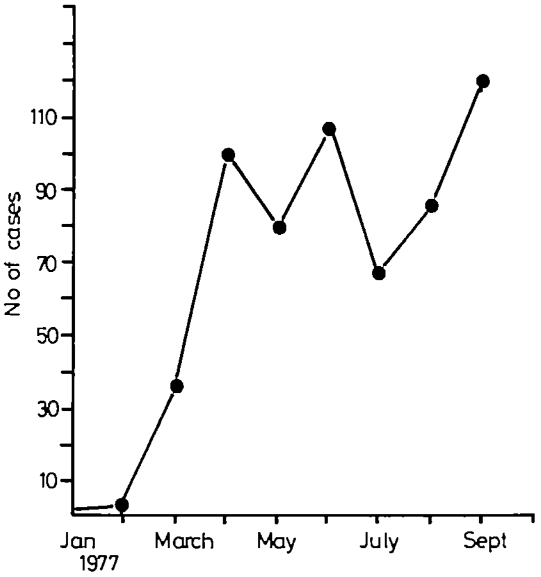


FIG 3 *Number of reported cases of chancroid in Greenland each month from 1 January to 1 October 1977*

In the first nine months of 1977, a total of 605 cases were reported—379 in men and 226 in women (a male-to-female ratio of 1·7 : 1). This ratio is much lower than that usually reported[7] and suggests equal partner change in men and women. The sex and age distribution per 1000 population is shown in Table I. Again, the high figures for girls aged 13-19 years are remarkable. In 1977, 975 cases were reported in Greenland. A drop in the monthly figures for chancroid started in November 1977 and continued in 1978; only 488 cases were reported in that year. A cause of the steep fall in the number of reported cases is undoubtedly due to the fact that the disease is very painful. A public debate on the disease and effective efforts to trace contacts has also been of importance.

Other sexually transmitted diseases

Lymphogranuloma venereum has not yet been encountered in Greenland, but nongonococcal urethritis, nongonococcal salpingitis, Reiter's disease, genital herpes, genital warts, and scabies are frequently seen.

Comments

The question may be asked why venereal diseases are so common in Greenland. During recent years, many factors have contributed to the spread of venereal diseases. The basic cause of the very high figures for venereal diseases is the social problems caused by change to a modern industrial society. The Greenlanders have not been given time to keep pace with developments; they have been mainly onlookers. This has given rise to problems of violence, promiscuity, and abuse of alcohol. Drinking is excessive; during recent years the annual consumption of alcohol has been well over 18 litres of 100% alcohol per person over 14 years of age. This year, politicians in Greenland have tried to curb the abuse of alcohol by introducing rationing. Among many other causes of the spread of venereal diseases is the concentration of the population, with many young people at a sexually active age, in the towns. This urbanisation has given rise to large youth groups, in which the individual members know less of the others than before, which makes the tracing of contacts difficult. The population over 13 years distributed by age group and by sex is given in Table II.[1] There are about 3500 more men than women, mainly due to the importation of manpower from Denmark. It is active men, mainly aged 20-44 years, who exert sexual pressure inevitably on the young Greenlander women.

All these factors are outside the influence of the national health service. During recent years, diagnostic aids have improved, but the health service may introduce further improvements by training personnel and by intensifing the dissemination of information on venereal diseases in the population. The appointment of a venereologist is required, not only for the solution of the many diagnostic and therapeutic problems but also to take care of the training, especially of Greenlander personnel. After training, these people must play an active part in health education, especially in schools and youth clubs. Well-trained Greenlander health workers, acting as "bare-foot doctors" under the guidance of the venereologist, can do a lot in the control of venereal diseases.

TABLE II *Age and sex distribution of population of 13 years or over. (In brackets persons not born in Greenland.)*

	Age group (years)			
Sex	*13-15*	*16-19*	*20-44*	*45 or over*
Male	1998 (121)	2233 (81)	11297 (4438)	3855 (781)
Female	2017 (123)	2057 (112)	8092 (1663)	3716 (172)

References

1. Groenlands Befolkning. Danmarks Statistik. Copenhagen, 1977.
2. Lange C. Bemaerkninger om Groenlands Sygdomsforhold. *Bibliot Laeger* 1864; **5RK8BD:** 15-64.
3. Berthelsen A. Groenlandsk medicinsk statistik og nosografi. In: Meddelelser om Groenland 1935-1943; Bd 117:III Copenhagen: Reitzel.
4. Olsen GA. Seksuel adfaerd blandt ungdom i Groenland. Copenhagen: Institut for Socialmedicin, Publication No 4, 1974.
5. Marcussen PV, Rendal J. A study of syphilis and sexual habits in Greenland. *Amer J Syph* 1950; **34:** 144-52.
6. Olsen GA. Syphilisepidemien i Sydgroenland i 1965. *Ugeskr Laeger* 1966; **128:** 1071-6.
7. Willcox RR. *A Textbook of Venereal Diseases*. London: William Heinemann, 1950.

Br J Vener Dis 1980;**56:**69-73.

Immunoglobulin-bearing lymphoid cells in primary syphilis

Quantitative and elution studies

J D BOS, F HAMERLINCK, AND R H CORMANE

From the Department of Dermatology, University of Amsterdam, Amsterdam, the Netherlands

SUMMARY The delay in antibody production in response to infection with *Treponema pallidum* may be caused by a block in the differentiation of antigen-stimulated B (Bursa-dependent) lymphoid cells towards plasma cells. This hypothesis was tested by a study to detect clonal expansion of immunoglobulin-bearing B lymphoid cells by in-vitro immunofluorescence tests in patients with primary syphilis. In addition, antibodies eluted from circulating lymphoid cells were investigated for treponemal binding by the enzyme-linked immunosorbent assay, the *T pallidum* immobilisation test, and the immunoglobulin class-specific FTA-ABS test.

Results indicated that the number of IgG-bearing lymphoid cells were increased in patients with primary syphilis. However, in only a few cases could antitreponemal antibodies be eluted from isolated lymphoid cells. For this reason, the original hypothesis was rejected.

Introduction

In syphilis the immunological response of the host to infection with *Treponema pallidum* has been extensively studied. As far as the cellular limb of immunity is concerned, many findings have pointed to impaired cell-mediated immunity in the early stages of infection.[1] Studies of the humoral response have shown that after infection immunoglobulins of different classes (IgG, IgA, IgM, and IgE) are extensively produced and their raised concentrations in serum and (at least partial) antitreponemal specificity have been demonstrated.[2-6]

Although *T pallidum* is present in the body after inoculation, there is often a delay before specific immunoglobulins can be detected. A temporary delay or block in the differentiation of antigen-stimulated B lymphoid cells into immunoglobulin-secreting plasma cells may be the origin of this delay.

The aim of the present study was to investigate this hypothesis. It was based on the previous findings of Cormane *et al*[7-9] that disease-specific antibodies could be eluted from circulating lymphoid cells in patients with autoimmune diseases such as lupus erythematosus, pemphigus, pemphigoid, scleroderma, and alopecia areata.

In this study the antitreponemal specificity of antibodies eluted from circulating lymphoid cells from patients with primary syphilis were investigated by the immunoglobulin-class-specific fluorescent treponemal antibody absorption (FTA-ABS) test, the *T pallidum* immobilisation (TPI) test, and the enzyme-linked immunosorbent assay (ELISA). At the same time, the number of immunoglobulin-bearing (B lymphoid) cells was estimated for each of the immunoglobulin subclasses by in-vitro immunofluorescence studies.[10]

Patients and methods

STUDY GROUP

The patients included in this study were referred from the outpatient department of our hospital. All were male, and a diagnosis of primary syphilis was based on the existence of an indurated ulcer from which *T pallidum* could be demonstrated by dark-field microscopy and a positive result to a treponemal serological test at the initial or at a subsequent examination.

A total of 12 patients was studied; of these, 10 were untreated at the time of investigation and two had been treated for less than one week. The time interval between infection and time of study varied from four to 12 weeks.

A complete study of sera and lymphoid cell eluates from 10 patients was carried out (Table I). In addition, B lymphoid cell subpopulations were quantitatively determined in 10 patients (Table II).

Address for reprints: Dr J D Bos, Department of Dermatology, University of Amsterdam, Binnengasthuis, Amsterdam, the Netherlands

Received for publication 9 July 1979

TABLE I *Results of serological tests performed on sera of 10 patients with primary syphilis*

	Serological test results									
						FTA-ABS test using				
Case No	*Kolmer*	*VDRL*	*RPCF*	*FTA-ABS*	*TPI*	*IgG*	*IgA*	*IgM*	*IgD*	*IgE*
1	−	+/−	+	+	−	+	−	+	−	+
2	−	−	−	+/−	−	+	+	+	−	+/−
3	−	+/−	−	+/−	−	+/−	−	+	−	−
4	+	+	+	+	+	+	−	−	−	+
5	−	+/−	−	+/−	−	+/−	−	−	−	+
6	+	+	+	+	+	+/−	−	+	−	+
7	−	−	−	+	−	+/−	−	−	−	−
8	+	+	+	+	+	+/−	−	+	−	+
9	+	+	−	+/−	−	+	−	+	−	+
10	−	+/−	+	+/−	−	−	−	+	−	−

+ Positive − negative +/− doubtful positive

TABLE II *Percentage of peripheral blood lymphocytes bearing surface immunoglobulin in 10 patients with primary syphilis*

		Percentage bearing immunoglobulins				
Case No	*Time after infection (weeks)*	*IgG*	*IgA*	*IgM*	*IgD*	*IgE*
1	7	9	0	5	3	2
2	6	25	2	15	5	4
4	6	11	1	5	4	1
5	NS	15	0	5	1	2
6	NS	25	2	10	10	7
7	4	7	3	2	1	2
8	4	4	1	2	2	2
9*	8	1	2	3	0	2
11	NS	25	2	8	6	11
12*	12	12	2	3	1	2
Range	4-12	1-25	0-3	2-15	0-10	1-11
Median		11·5	2·0	5·0	2·5	2·0
Standarised W test		2·6537	0·7177	0·0000	−0·6070	1·3384
P		0·0089	0·4979	1·0304	0·5693	0·1943

NS = Not specified
*Patients treated for less than one week

CONTROLS

Ten healthy staff members served as controls for the quantitative determination of B lymphoid cells. All were male and of the same age range as the study group.

SEROLOGICAL TESTS

The FTA-ABS test* was performed according to the standard method described by Kawamura *et al.*[11] It was modified as described before.[6]

The Treponematoses Department of the National Institute of Public Health (NIPH) investigated all sera with the Kolmer modification of the Wassermann reaction, the Venereal Disease Research Laboratory (VDRL) test, the Reiter protein complement fixation (RPCF) test, the FTA-ABS test, and the TPI test. Lymphoid cell eluates were investigated with the TPI and ELISA tests.

ISOLATION OF LYMPHOID CELLS

Lymphoid cell separation was carried out according to the method of Böyum,[12] with some minor modifications as described below.

Fifty millilitres of peripheral blood was collected by venepuncture in Erlenmeyer bats containing 50 glass beads and shaken for 10 minutes for defibrination. To this, 70 ml of phosphate-buffered saline (PBS), pH 7·4, was added. Portions of 40 ml of this suspension were layered on top of portions of 10 ml Ficoll-Isopaque mixture. The suspensions were centrifuged at 400 × *g* for 20 minutes. The resulting lymphoid-cell-rich layer between Ficoll-Isopaque and supernatant was aspirated and washed three times in PBS. After each washing, the lymphoid cell suspension was centrifuged at 200 × *g* for five minutes. The cell layer obtained was composed of 90-95% lymphoid cells and 5-10% monocytes and neutrophils. Viability of the isolated cells was over 98% as determined by trypan blue exclusion. The last washing solution served as control for subsequent immunoglobulin studies.

---

* Specifications of the antisera used may be obtained from the authors.

ELUTION OF IMMUNOGLOBULINS

The lymphoid cells were resuspended in 0·5 ml of a 0·05 mol/l citrate buffer (pH 3·2) and incubated overnight at 4°C. After incubation, the suspension was centrifuged at 200 × *g* for 10 minutes and the supernatant (now called eluate) brought to a pH of 7·2 by addition of 0·1 mol/l NaOH.

Without dialysis or concentration the eluate was tested for the presence of antitreponemal immunoglobulins: (a) undiluted with class-specific FTA antisera; (b) diluted 1/2 in sorbent with class-specific FTA antisera; (c) diluted 1/12 by the TPI test; and (d) diluted 1/10 and 1/100 by ELISA.

IMMUNOFLUORESCENCE STUDIES

Fluorescein isothiocyanate-(FITC)-labelled antisera directed against human IgG, IgA, IgM, IgD, and IgE were obtained from Meloy Laboratories (Springfield, Illinois).*

Direct staining of isolated lymphoid cells in suspension was carried out by suspending 0·1 ml washed viable cells (2 × 10^5) in 0·1 ml of each conjugate (final conjugate dilution 1/40). This mixture was then incubated at room temperature for 30 minutes. After incubation the cells were washed twice for five minutes at 200 × *g* in PBS and resuspended in 0·2 ml PBS.

In each case, the percentage of IgG-, IgA-, IgM-, IgD-, and IgE-positive cells was determined by examining the microscopic field alternately in blue narrow-band epi-illumination (480-490 nm) for fluorescence and in conventional light.

FLUORESCENT MICROSCOPY

A Leitz Orthoplan microscope with a Xenon XBO-75 lamp and a Leitz vertical illuminator was used. The primary filter combination was BG 38 (4 mm), KP 490 (2 mm), GG 475 (2 mm), and the secondary filter was an OG 513 (3 mm). For routine use, examination was performed with a × 10 ocular and a Leitz × 40 dry objective.

PERIPHERAL BLOOD COUNTS

Total peripheral leucocyte counts and their differentiation were estimated to relate percentage figures to the absolute numbers of lymphoid cells.

STATISTICAL METHOD

The non-parametric Wilcoxon's test,[13] with the usual correction for ties and the normal approximation to obtain the P value, was used for statistical analysis of the numbers of immunoglobulin-bearing lymphoid cells in patients with primary syphilis and in healthy controls.

TEST MATERIALS

Isopaque (Natrii N-, ethyl-3,5-diacetamido-2.4.6.-trijodbenzoas) solution: 20 ml Isopaque (Nyegaard and Co, Oslo, Norway) was diluted with 25 ml distilled water.

Isopaque-Ficoll mixture: 10 parts Isopaque solution were mixed with 24 parts of Ficoll (9%) (Pharmacia, Uppsala, Sweden).

Citrate buffer, pH 3·2, 0·05 mol/l: 43·7 ml 0·1 mol/l citric acid + 6·3 ml 0·1 mol/l sodium citrate diluted with demineralised water to 100 ml.

0·1 mol/l citric acid: 21·02 *g* citric acid monohydrate diluted with demineralised water to 1 litre.

0·1 mol/l sodium citrate: the dihydrate rather than the pentahydrate should be used: 29·4 *g* of trisodium citrate dihydrate dissolved in CO_2-free water, and the volume made up to 1 litre.

pH indicator paper pH 6·4-8·0 was obtained from E Merck, Darmstadt, W Germany.

Trypan blue: 0·05% in PBS.

Results

SEROLOGICAL TESTS

Results of serological tests on the sera of cases 1-10, performed in the NIPH and in our own laboratory, are given in Table I. Since it was the aim of this study to investigate patients in the earliest stage of primary syphilis, all results are presented. From the results of tests performed by the reference laboratory it may be concluded that three patients (cases 2, 3, and 5) were sero-negative at the time of investigation as they had only doubtful VDRL or FTA-ABS test results or both. However, two patients (cases 2 and 3) had positive results to the IgM-FTA-ABS test in our own laboratory, which may not have been detected by the anti-Ig (anti-total) conjugate used in the NIPH. Only two patients (cases 4 and 8) were unequivocally seropositive.

B LYMPHOID CELL DETERMINATION

The results of B lymphoid cell determination in 10 patients with primary syphilis are given in Table II and those of 10 healthy controls in Table III. Absolute numbers of lymphoid cells in the study group did not differ from normal (mean: 2·35 × 10^9/l: range 1·66-2·95 × 10^9/l), and statistical analysis was performed on percentage figures.

---

* Specifications and working dilutions of antisera used for immunofluorescence studies may be obtained from the authors.

TABLE III *Percentage of peripheral blood lymphocytes bearing surface immunoglobulins in 10 healthy controls*

	Percentage bearing immunoglobulins				
Control No	*IgG*	*IgA*	*IgM*	*IgD*	*IgE*
1	2	0	8	6	2
2	2	0·5	1	1·5	1
3	7	2	8	8	3
4	3	1	6	4	5
5	3	0·5	3	3·5	1·5
6	2·5	2	6	3	1
7	3·5	1·5	3	1·5	2
8	4	1	2	2·5	2
9	8	2	7	2	1
10	6	2	6	2·5	2
Range	2-8	0-2	1-8	1·5-8	1-5
Median	3·25	1·2	5·0	3·5	2

The exact values of standarised W and two-sided tail probability, obtained by applying Wilcoxon's test to each of the five investigated immunological traits, are given in Table II. The study group and the control group differed significantly in the number of IgG-bearing lymphoid cells (P 0·0089). The number of IgG-bearing lymphoid cells had increased in the study group, its median being 8·25% higher than that of the control group. No significant difference was found in the other subclasses of immunoglobulin-bearing lymphoid cells.

ELUTION STUDIES

In only two cases was a clearly positive IgM-FTA test result obtained with undiluted eluates of isolated lymphoid cells. Of these, one patient (case 1) continued to have a positive result, and the result of another (case 8) became negative after 1/2 dilution of eluate in sorbent (IgM-FTA-ABS test). Borderline FTA test results (undiluted) were obtained twice for IgG (cases 2 and 8), once for IgM (case 2), and once for IgD (case 3). The results of the IgA-FTA and IgE-FTA tests, ELISA, and TPI test were all negative as were the findings in the last washing solutions.

Discussion

The results of determining the numbers of immunoglobulin-bearing lymphoid cells in primary syphilis indicate that the number of IgG-bearing lymphoid cells are significantly increased, with a probability of $P=0 \cdot 0089$.

In the study group, circulating antitreponemal IgM antibodies were detected in five out of eight patients, the remaining three probably having false-negative results. The production of circulating antitreponemal IgG seemed to be only beginning, as the results in four out of eight cases to the IgG-FTA-ABS test were positive and those in the other four were borderline (Tables I and II).

The initiation of antitreponemal IgG production—with intervals between the date of infection and the time of investigation varying from four to 12 weeks—and the possible clonal expansion of IgG-bearing lymphoid cells seemed to confirm that a block in the differentiation of antigen-stimulated B lymphoid cells towards secreting plasma cells might be responsible for the delay in detectable antibody production in early syphilis. However, the results of the serological tests (TPI, ELISA, class-specific FTA and FTA-ABS tests) made us reject that hypothesis. No group-specific or species-specific antitreponemal antibodies could be demonstrated consistently in the eluates, not even from increased numbers of circulating IgG-bearing lymphoid cells, indicating that these were not precursors for antitreponemal antibody-producing plasma cells.

Thus, the increased number of IgG-bearing lymphoid cells in primary syphilis, as detected by surface membrane Ig-marking, may have originated from another immunological phenomenon.[10] Firstly, the technique may have detected IgG-treponemal antigen immune complexes bound via Fc receptors to non-B lymphoid cells.[14] Secondly, anti-lymphocyte autoantibodies of the IgG-class may have been detected by the test procedure. Although these antibodies have not as yet been detected in syphilis, a T cell inhibitory factor is known to exist in syphilitic sera.[1 15]

The rejection of the original hypothesis, and thus the probability of normal B lymphoid cell differentiation in primary syphilis, is in agreement with the role of the extracellular material at the surface of *T pallidum*.[16 17] This layer may not only prevent non-immunological defence mechanisms from interfering with the invading micro-organism but may also cause a delay in antibody production as a consequence of its low antigenic capacity. Moreover, it may explain the limited success of in-vivo passive immunisation with syphilitic sera in rabbit experiments.[18 19] The finding of increased numbers of IgG-bearing lymphoid cells in patients with primary syphilis needs to be confirmed but it may be definitively explained by future investigations.

We wish to thank all our colleagues for their referral of the patients. This investigation was made possible by a grant from the Prevention Fund (The Hague, the Netherlands). We are also much indebted for the technical advice and support from G Aalbers, P M Lansdorp, H E Menke, and J J Snooijink. Statistical analysis was performed at the Mathematical Centre, Amsterdam by R Gill. The Treponematoses Department of the National Institute of Public Health (Dr H E Menke) gave us their full co-

operation in this study and kindly performed the serological tests.

References

1. Pavia CS, Folds JD, Baseman JB. Cell-mediated immunity during syphilis. *Br J Vener Dis* 1978; **54**: 144-50.
2. Delhanty JJ, Catterall RD. Immunoglobulins in syphilis. *Lancet*, 1969; **2**: 1099-103.
3. Menke HE. Immunglobulinen en thymoltroebeling bij vroege syphilis. *Thesis*, Rotterdam: Erasmus Universiteit, 1975.
4. Green RL, Scales RW, Kraus SJ. Increased immunoglobulin E concentrations in venereal diseases. *Br J Vener Dis* 1976; **52**: 257-60.
5. Julian AJ, Logan LC, Norins LC. Early syphilis: immunoglobulins reactive in immunofluorescence and other serologic tests. *J Immunol* 1969; **102**: 1250-9.
6. Bos JD, Hamerlinck F, Cormane RH. Antitreponemal IgE in early syphilis. *Br J Vener Dis* 1980; **56**: 20-25.
7. Cormane RH, Hamerlinck F, Husz S. Elution of antibodies from the lymphocyte membrane in certain dermatoses. *Br J Dermatol* 1974; **91**: 315-6.
8. Cormane RH, Hamerlinck F, Nunzi E. Antibodies eluted from the lymphoid cell membrane: occurrence in certain varieties of scleroderma. *Arch Dermatol* 1979; **115**: 709-12.
9. Cormane RH, Hamerlinck F, Nunzi E. Antibodies against endothelia. In: Wolff K, ed. *Proceedings of the International Symposium on Vasculitis* (in press).
10. WHO/IARC Workshops. Identification, enumeration, and isolation of B and T lymphocytes from human peripheral blood. *Scand J Immunol* 1974; **3**: 521-32.
11. Kawamura A, Mizuoka K, Matuhasi T, Fukuoka Y. Methods used in Japan for FTA-ABS tests (fluorescent treponemal antibody test). In: Beutner EH *et al*, eds. *Immunopathology of the Skin: Labeled Antibody Studies*. Stroudsberg, Pennsylvania USA: Dowden, Hutchinson and Ross, 1973; 425-45.
12. Bøyum A. Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968; **21**, suppl 97: 1-106.
13. Siegel S. *Nonparametric Statistics*. New York: McGraw-Hill, 1956.
14. Alexander EL, Henkart PA. Human peripheral lymphocytes bearing surface immunoglobulin do not have readily detectable Fc receptors. *Clin Exp Immunol* 1978; **33**: 332-9.
15. Levene GM, Turk JL, Wright DJM, Grimble AGS. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. *Lancet* 1969; **2**: 246-7.
16. Christiansen S. Protective layer covering pathogenic treponemata. *Lancet* 1963; **1**: 423-5.
17. Zeigler JA, Jones AM, Jones RH, Kubica KM. Demonstration of extracellular material at the surface of pathogenic *Treponema pallidum* cells. *Br J Vener Dis* 1976; **52**: 1-8.
18. Weiser RS, Ericson D, Perine PL, Pearsall NN. Immunity to syphilis: passive transfer in rabbits using serial doses of immune serum. *Infect Immun* 1976; **13**: 1402-7.
19. Bishop NH, Miller JN. Humoral immunity in experimental syphilis. I The demonstration of resistance conferred by passive immunization. *J Immunol* 1976; **117**: 191-6.

Br J Vener Dis 1980;**56**:74-6.

T lymphoid cells in primary syphilis

Quantitative studies

J D BOS, F HAMERLINCK, AND R H CORMANE

From the Department of Dermatology, University of Amsterdam, Amsterdam, the Netherlands

SUMMARY In previous studies to assess the role of cell-mediated immunity in *Treponema pallidum* infection investigations of delayed type skin hypersensitivity tests, lymphocyte transformation test, leucocyte migration inhibition tests, and histological studies of the reticulo-endothelial system have been performed.

In this study, the numbers of T (thymus-dependent) lymphoid cells in 10 patients with primary syphilis (eight untreated) were estimated by the rosette technique. Results indicate a significant decrease in the number of T lymphoid cells in patients with primary syphilis.

Introduction

The results of studies to assess the role of cell-mediated immunity in *Treponema pallidum* infection are inconsistent and therefore difficult to interpret, although the general impression that cell-mediated immunity has a protective function in syphilis—but is somehow adversely affected during the early and infectious stages of the disease—still remains.

Studies of delayed type skin hypersensitivity towards *T pallidum* antigens[1] indicate that a specific anergy to antigens of *T pallidum* exists in the primary and secondary stage, as well as in general paralysis of the insane, without impairment of responses to other unrelated antigens.

The lymphocyte transformation test in human and experimental rabbit syphilis has been studied,[2-16] but the results are conflicting; in some studies, a normal lymphoblastic response to T-cell mitogen phytohaemagglutinin (PHA), but reduced proliferation to treponemal antigens without suppression by syphilitic sera, was observed,[7 8] whereas in other studies a diminished lymphoblastic response to T-cell mitogens (concanavalin-A (con-A) and PHA) but normal proliferation to *T pallidum* antigens was found, although the latter could be suppressed by syphilitic sera.[3 9-13 15]

The results of studies using the leucocyte migration inhibition test[10 17-20] are more consistent and agree with the results of delayed type skin hypersensitivity tests, indicating a diminished cell-mediated immunity towards *T pallidum* in primary and secondary syphilis.

Investigations of the histological appearance of lymphoid tissues in syphilis have shown that thymus-dependent areas are adversely affected in early acquired syphilis and in congenital syphilis.[21 22]

Another method of assessing the cellular limb of immunity in syphilis is by determining the number of circulating T lymphoid cells in relation to the general distribution of lymphoid cells in patients with syphilis. In the present study, the number of circulating T lymphoid cells was estimated in 10 patients with primary syphilis by the rosette technique[23] and compared with that in a healthy control group.

Patients and methods

STUDY GROUP

The patients included in this study were referred from the outpatient department of our hospital. A total of 10 patients was studied (the same as in a previous study[24]).

All patients were male, and a diagnosis of primary syphilis was based on the existence of an indurated ulcer from which *T pallidum* could be demonstrated by darkfield microscopy and on a positive result to a treponemal serological test at the initial or at a subsequent examination.

Eight of the patients were untreated at the time of investigation whereas two had been treated for less than one week.

HEALTHY CONTROLS

Ten healthy staff members served as controls for the quantitative determination of T lymphoid cells. All were male and of the same age range as the study group.

Address for reprints: Dr J D Bos, Department of Dermatology, University of Amsterdam, Binnengasthuis, Amsterdam, the Netherlands

Received for publication 21 August 1979

ISOLATION OF LYMPHOID CELLS

Lymphoid cell separation was carried out as described previously[24] according to the method of Böyum,[25] with some minor modifications as described below.

Ten millilitres of peripheral blood was collected aseptically by venepuncture in Erlenmayer bats containing 10 glass beads and shaken for 10 minutes for defibrination. To this was added 30 ml of phosphate-buffered saline (PBS). Portions of 10 ml of this suspension were layered on top of portions of 2·5 ml Ficoll-Isopaque mixture and centrifuged at 400 × *g* for 10 minutes. The resulting lymphoid-cell-rich layer between the Ficoll-Isopaque and the supernatant was aspirated and washed three times in PBS. After each washing, the lymphoid-cell suspension was centrifuged at 200 × *g* for five minutes. The lymphoid-cell-rich layer was composed of about 90-95% lymphoid cells and 5-10% monocytes and neutrophils. Viability of the isolated cells was over 98% as determined by trypan blue exclusion.

DETERMINATION OF T LYMPHOID CELLS

Isolated lymphoid cells (1 × 10^6) were resuspended in 0·3 ml minimal essential medium (MEM); to this was added 0·3 ml of fresh sheep red blood cell (SRBC) suspension containing a total of 50 × 10^6 SRBC: this suspension of SRBC had previously been washed four times in MEM, each time at 200×*g* for 10 minutes. The whole suspension of lymphoid cells and SRBC was centrifuged at 200 × *g* for five minutes and then incubated for one hour at 4°C. It was resuspended carefully. Only those lymphoid cells which had attracted at least three SRBC to form a rosette were counted (by light microscopy); these were T lymphoid cells and their number was expressed as a percentage of the total lymphoid cells in suspension.

PERIPHERAL BLOOD COUNTS

Total peripheral leucocyte counts and their differentiation were estimated to relate percentage figures to the absolute numbers of lymphoid cells.

STATISTICAL METHOD

The non-parametric Wilcoxon's test,[26] with the usual correction for ties and the normal approximation to obtain the P value, was used for statistical analysis of the numbers of T lymphoid cells in the study group and in controls.

MATERIALS

Isopaque (Natrii N-, ethyl-3,5-diacetamido-2.4.6-trijodbenzoas) solution: 20 ml Isopaque (Nyegaard and Co, Oslo, Norway) was diluted with 25 ml distilled water.

Isopaque-Ficoll mixture: 10 parts Isopaque solution were mixed with 24 parts of Ficoll (9% in distilled water) (Pharmacia, Uppsala, Sweden).

Phosphate-buffered saline (PBS), pH 7·4: 8·2 g NaCl + 1·6 g $Na_2HPO_4.2H_2O$ + 0·2 g $NaH_2PO_4.2H_2O$ dissolved in 1 litre distilled water.

Minimal essential medium (MEM): A modified Eagle's solution containing 2 g/l sodium bicarbonate without glutamine (Flow Laboratories, Irvine, Scotland).

Sheep red blood cells (SRBC): Fresh sheep red blood cell suspension in Alsevers medium (Central Laboratory of the Red Cross Blood Transfusion Services, Amsterdam, the Netherlands).

Trypan blue: 0·05% in PBS.

Results

The results of T lymphoid cell determination in 10 patients with primary syphilis are given in the Table, together with those in the 10 healthy controls. Absolute numbers of lymphoid cells in the study group did not differ from normal (mean: 2·35 × 10^9/l; range 1·66-2·95 × 10^9/l), and statistical analysis was performed on percentage figures.

TABLE *Percentages of T lymphoid cells in 10 patients with primary syphilis and in 10 healthy controls*

Case No	*T cells (%)*	*Control No*	*T cells (%)*
2	45	1	62
3	63	2	63
4	50	3	62
5	53	4	69
6	50	5	61·5
7	51	6	65·5
8	63	7	59
9*	53	8	54·5
11	49	9	64
12*	53	10	65
Range	45-63	Range	54·5-69·0
Median	52·0	Median	62·5

*Patients who had been treated for less than a week
Standardised W = −2·9593
P value = 0·0035

The exact values of standardised W and two-sided tail probability, after Wilcoxon's test had been applied to the percentages of T lymphoid cells, are given in the Table. The number of T lymphoid cells in the study group and the control group was significantly different (P = 0·0035), with a difference in medians of −10·5%, indicating a decrease in T lymphoid cells in primary syphilis.

Discussion

In this study, the evidence suggests that the number of T lymphoid cells in patients with primary syphilis is decreased; only two out of 10 had normal percentages (cases 3 and 8). This decrease in the number of T cells is associated with an increase in IgG-bearing lymphoid cells found in the same patients.[24] Thus the selective depletion of T lymphoid cells in the paracortical areas of lymph nodes from patients with primary syphilis[21] seems not to be accompanied by an increase in the number of circulating T cells. It is uncertain whether or not the decrease in circulating T cells is another effect of the serum factor(s) that depress the lymphoblastic response to *T pallidum* antigens of isolated lymphoid cells, especially enriched T cell populations.[13]

The exact nature of the suppressing factor(s) in syphilitic sera is still unknown; they may be of treponemal origin (soluble antigens) or there may be a shift in the homeostasis of soluble mediators of immune regulation.[27] In this respect it is interesting that the production of lymphocyte mitogenic factor was impaired in lymph node cells of rabbits infected with *T pallidum*.[16]

Decreased concentrations of circulating T cells in primary syphilis is certainly not disease-specific; these have been found in a wide variety of diseases, infectious as well as non-infectious.[28] This study provides no information on the exact nature of the decrease in T cells—whether there is a selective depletion of a subclass of T cells or whether the decrease is the result of an interaction of serum factor(s) on the physicochemical binding of T cells and SRBC with or without impairment of immune function. Studies are at present being directed towards the elucidation of some of these possibilities.

We wish to thank all our colleagues for referring their patients. This investigation was made possible by a grant from the Prevention Fund, The Hague, the Netherlands. We are also much indebted for the technical advice and support of P M Lansdorp and H E Menke. Statistical analysis was performed by R Gill at the Mathematical Centre, Amsterdam, the Netherlands.

References

1. Wright DJM, Grimble AS. Why is the infectious stage of syphilis prolonged? *Br J Vener Dis* 1974; **50:** 45-9.
2. Badanoiu A, Gavrilesco M, Nicolau G, Circiumaresco T. Lymphoblastogenèse immunoallergique et antigène tréponémique in vitro dans la syphilis. *Arch Roum Path Exp Microbiol* 1969; **28;** 419-27.
3. Levene GM, Turk JL, Wright DJM, Grimble AGS. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. *Lancet* 1969; **2:** 246-7.
4. Janot C, Grandidier M, Pupil P, Thomas J-L, Beurey J, de Lavergne E. Le test de transformation lymphoblastique au cours de la syphilis. *Presse Médicale* 1971; **79:** 1901-4.
5. Friedmann PS, Turk JL. A spectrum of lymphocyte responsiveness in human syphilis. *Clin Exp Immunol* 1975; **21:** 59-64.
6. Friedmann PS, Turk JL. The role of cell-mediated immune mechanisms in syphilis in Ethiopia. *Clin Exp Immunol* 1978; **31:** 59-65.
7. Musher D, Schell R, Knox JM. In-vitro lymphocyte response to *Treponema refringens* in human syphilis. *Infect Immun* 1974; **8:** 654-7.
8. Musher D, Schell RF, Jones RH, Jones AM. Lymphocyte transformation in syphilis: an in-vitro correlate of immune suppression in vivo? *Infect Immun* 1975; **11:** 1261-4.
9. Kantor FS. Infection, anergy and cell-mediated immunity. *N Engl J Med* 1975; **292:** 629-34.
10. From E, Thestrup-Pedersen K, Thulin H. Reactivity of lymphocytes from patients with syphilis towards *T pallidum* antigen in the leucocyte migration and lymphocyte transformation tests. *Br J Vener Dis* 1976; **52:** 224-9.
11. Pavia CS, Folds JD, Baseman JB. Depression of lymphocyte response to concanavalin-A in rabbits infected with *Treponema pallidum* (Nichols strain). *Infect Immun* 1976; **14:** 320-2.
12. Pavia CS, Baseman JB, Folds JD. Selective response of lymphocytes from *Treponema pallidum* infected rabbits to mitogens and *Treponema reiteri*. *Infect Immun* 1977; **15:** 417-22.
13. Pavia CS, Folds JD, Baseman JB. Selective in-vitro response of thymus-derived lymphocytes from *Treponema pallidum* infected rabbits. *Infect Immun* 1977; **18:** 603-11.
14. Wicher V, Wicher K. In-vitro cell response of *Treponema pallidum*-infected rabbits. I Lymphocyte transformation. *Clin Exp Immunol* 1977; **29:** 480-6.
15. Wicher V, Wicher K. In-vitro cell response of *Treponema pallidum*-infected rabbits. II Inhibition of lymphocyte response to phytohaemagglutinin by serum of *Treponema pallidum*-infected rabbits. *Clin Exp Immunol* 1977; **29:** 487-95.
16. Wicher V, Wicher K. In-vitro cell response of *Treponema pallidum*-infected rabbits. III Impairment in production of lymphocyte mitogenic factor. *Clin Exp Immunol* 1977; **29:** 496-500.
17. Fulford KWM, Brostoff J. Leucocyte migration and cell-mediated immunity in syphilis. *Br J Vener Dis* 1972; **48:** 483-8.
18. Wicher V, Wicher K. Cell response in rabbits infected with *Treponema pallidum* as measured by the leucocyte migration inhibition test. *Br J Vener Dis* 1975; **51:** 240-5.
19. Metzger M, Podwinska J, Smogor W. Cell-mediated immunity in experimental syphilis in rabbits. *Arch Immun Therap Exp* 1977; **25:** 25-34.
20. Pavia CS, Baseman JB, Folds JD. Development of macrophage migration inhibition in rabbits infected with virulent *Treponema pallidum*. *Infect Immun* 1977; **17:** 651-4.
21. Turner DR, Wright DJM. Lymphadenopathy in early syphilis. *J Path* 1973; **110:** 305-8.
22. Levene GM, Wright DJM, Turk JL. Cell-mediated immunity and lymphocyte transformation in syphilis. *Proc Roy Soc Med* 1971; **64:** 426-8.
23. WHO/IARC Workshop: Identification, enumeration, and isolation of B and T lymphocytes from human peripheral blood. *Scand J Immunol* 1974; **3:** 521-32.
24. Bos JD, Hamerlinck F, Cormane RH. Immunoglobulin-bearing lymphoid cells in primary syphilis. *Br J Vener Dis* 1980; **56:** 69-73.
25. Böyum A. Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968; **21** Suppl **97:** 1-106.
26. Siegel S. *Nonparametric Statistics*. New York: McGraw-Hill, 1956.
27. Waksman BH, Namba Y. On soluble mediators of immunologic regulation. *Cell Immunol* 1976; **21:** 161-76.
28. Wybran J, Fudenberg HH. Thymus-derived rosette-forming cells in various human disease states: cancer, lymphoma, bacterial and viral infections, and other diseases. *J Clin Invest* 1973; **52:** 1026-32.

Br J Vener Dis 1980;**56**:77-80.

Further evaluation of the microhaemagglutination test to determine treponemal antibodies in CSF

S KASATIYA AND A BIRRY

From the Ontario Public Health Laboratory, Ottawa, Ontario; and the Laboratory Services, Quebec Department of Social Affairs, Ste-Anne de Bellevue, Canada

SUMMARY The results of a microhaemagglutination test (MHA:TP) using diluted (MHA:TP-DCSF) and undiluted (MHA:TP-UCSF) samples of cerebrospinal fluid (CSF) from 1452 patients with syphilis, neurological, metabolic, or immunological disorders, and undetermined diagnoses to detect antitreponemal antibodies were compared with those of the Venereal Disease Research Laboratory (VDRL-CSF) test and the fluorescent treponemal antibody (FTA-CSF) test. Using undiluted samples of CSF the MHA:TP test gave more reactive results than with diluted samples. The MHA:TP-UCSF test was slightly more specific and sensitive than the FTA-CSF test. The use of undiluted CSF did not reduce the specificity of the MHA:TP test, as was shown by testing CSF samples from patients with diseases other than syphilis.

Introduction

More and more laboratories, because of high costs, use tests which are simple, rapid, economical, sensitive, and specific. The *Treponema pallidum* haemagglutination (TPHA) test[1] has been used frequently for determining treponemal antibodies using serum. This test, using samples of cerebrospinal fluid (CSF), has been evaluated by only a few workers,[2-4] mostly because few samples of normal and abnormal CSF are available as physicians are reluctant to perform lumber punctures, which may have serious sequelae.

The methods[5 6] described for the micro-automated version of the TPHA (MHA:TP) test are used for serum testing. As the concentration of immunoglobulins in CSF is far lower than in the serum, it was presumed that this might affect the reactivity of the MHA:TP test.

The present study of the MHA:TP test using diluted (MHA:TP-DCSF) and undiluted (MHA:TP-UCSF) CFS was prompted by the finding of a remarkable difference in reactivity with diluted and undiluted CSF during a previous study.[2]

Patients and methods

SPECIMENS

During the period from September 1978 to March 1979, specimens of CSF from 1452 patients were received at the central public health laboratory. Of these, 210 came from known cases of syphilis, based on serological results and clinical and historical information, and the remaining 1242 from patients with undetermined diagnoses or with neurological, metabolic, or immunological disorders. Specimens were collected in the local hospitals and brought to the laboratory by messenger within 24 hours. The specimens were kept at −20°C until tested.

TESTS

Although the main purpose of the study was to evaluate the MHA:TP test using CSF, additional tests were performed to verify the previous results.[2 7 8] The Venereal Disease Research Laboratory (VDRL-CSF) test[9] and the fluorescent treponemal antibody (FTA-CSF) test[8 10] were performed on undiluted unabsorbed CSF. Antigens, reagents, and controls for the FTA-CSF test were supplied by Beckman Company (Fullerton, California). For routine testing, monospecific IgG conjugate was used in a dilution of 1/2300 to obtain the same intensity of fluorescence with strongly reactive, weakly reactive, and non-reactive CSF controls selected in the laboratory. Monospecific IgM conjugate was supplied by Wellcome Research Laboratories (Beckenham, Kent). A Leitz Orthoplan microscope equipped with an incident light system was used.[11]

The MHA:TP test was performed on uninactivated CSF diluted 1/20 in absorption buffer[2 6] and on uninactivated undiluted unabsorbed CSF. Antigens, absorption buffer, and serum controls for this test were supplied by the Fujizoki Pharmaceutical Company (Tokyo, Japan).

Address for reprints: Dr S Kasatiya, Ontario Public Health Laboratory, Box 6301, Station J, Ottawa, Ontario, Canada K2A 1S8

Received for publication 30 April 1979

Results

REACTIVITY IN CSF

Known and unknown diseases

Of 1452 specimens, 7·6% were reactive to the MHA:TP-DCSF test compared with 9·7% to the MHA:TP-UCSF test; 89·3% were not reactive to any of the tests whereas 2·8% were reactive to all four tests (Table I). All the 41 specimens reactive to the four tests came from known cases of syphilis. Interestingly, 14 (1%) specimens were reactive to the MHA:TP-UCSF test and not to the FTA-CSF test, whereas the contrary was true for a further 14 (1%) specimens. Of these 28 specimens, 24 came from known cases of syphilis and four (reactive only to the FTA test) from patients with diseases other than syphilis (Table II).

Known cases of syphilis

The reactivity of the four tests with CSF from 210 known cases of syphilis are shown in Table III. The MHA:TP-UCSF test was the most sensitive of all the tests, being reactive with 67·1% of the specimens; 19·5% of specimens were reactive to all four tests. The percentage reactivity of the FTA-CSF test was midway between that of the MHA:TP-DCSF and the MHA:TP-UCSF tests. In 17 (8·1%) cases, the MHA:TP-DCSF test was non-reactive whereas both the MHA:TP-UCSF and FTA-CSF tests were reactive.

Primary syphilis

All the CSF samples from 17 known cases of primary syphilis were non-reactive to the four tests.

Secondary syphilis

A good correlation in percentage reactivity and agreement between the MHA:TP-UCSF and FTA-CSF tests was obtained in 27 cases of secondary syphilis. Eighteen (66·6%) of the specimens were reactive to both tests. The MHA:TP-DCSF test detected 18% fewer reactive results than either of the previous two tests. One CSF sample from a case of secondary syphilis was reactive to the MHA:TP-UCSF test whereas another case was reactive to the FTA-CSF test alone.

Tertiary syphilis

The MHA:TP-UCSF and the FTA-CSF tests were equally sensitive, being reactive in 75 (90·4%) samples of CSF from 83 patients with tertiary syphilis. An agreement of 100% was obtained between the MHA:TP-UCSF and the FTA-CSF tests. Almost twice as many samples from cases of tertiary syphilis were reactive to all four tests than were cases of secondary syphilis. The non-reactive samples decreased from 33·4% in secondary to 9·6% in tertiary syphilis. The MHA:TP-DCSF test was 10% less sensitive than the MHA:TP-UCSF test.

Latent syphilis

As expected, all four tests gave fewer reactive results for CSF samples from cases of latent syphilis. Only five (6·5%) of 77 samples were reactive whereas 23 (29·9%) were non-reactive to all four tests. The FTA-CSF test was slightly more sensitive than the MHA:TP-DCSF test but less sensitive than the MHA:TP-UCSF test. Samples from 12 (15·6%) cases of latent syphilis were reactive to the latter test only compared with eight (10·4%) to the FTA-CSF test alone. The FTA-CSF test also gave non-reactive results for one specimen which was reactive to the remaining three tests.

Diseases other than syphilis

No reactivity to the MHA:TP-UCSF test was obtained with samples from 247 patients with diseases other than syphilis. A reactive result to the FTA-CSF test was obtained for four samples, one each from a case of vertebral disc protrusion, a case of lumbar radiculopathy, a neurological case, and a case of Wernicke Korsakoff's disease with a past history of primary syphilis 27 years previously.

Discussion

As expected, most of the data support our previous findings[2 8] on the sensitivity and specificity of the VDRL-CSF, MHA:TP-CSF and FTA-CSF tests in detecting antitreponemal antibodies in CSF. The MHA:TP-CSF test performed on undiluted CSF samples gave more reactive results than on diluted samples. Dilution of the CSF reduces the total quantity of the immunoglobulins available, thus diminishing the visible reaction in the microhaemagglutination test. The loss of reactivity to the FTA-CSF test was obtained by Jaffe *et al*[3] by performing the test on diluted samples of CSF.

Cases of latent syphilis are the most difficult to confirm, particularly by examination of the CSF, which makes a misdiagnosis quite likely. Of the 24 samples of CSF where there was disagreement between the three tests, 14 were reactive to the MHA:TP-UCSF and non-reactive to the FTA-CSF tests. Of the 14, 13 were cases of latent and one of secondary syphilis. This disagreement may indicate either that the diagnosis of latent syphilis in these cases was wrong or that the test gave a false-positive result with undiluted CSF or, more probably, that they were in fact cases of old or recent syphilis. A reactive result to the haemagglutination test, even in primary syphilis, when the results of the FTA test are non-reactive, should be cautiously interpreted.[12]

TABLE I *Comparative results of the VDRL-CSF, MHA:TP-DCSF, MHA:TP-UCSF and FTA-CSF tests on specimens of CSF from 1452 cases*

	Tests				Total		Agreement between tests (%)				
	VDRL	MHA-D	MHA-U	FTA	No	%	VDRL: MHA-D	VDRL: MHA-U	VDRL: FTA	MHA-D: FTA	MHA-U: FTA
	+	+	+	+	41	2·8					
	−	+	+	+	69	4·8					
								1353 (93·2)		1420 (97·8)	
	−	−	+	+	17	1·2					
							1383 (95·3)		1351 (93·0)		1424 (98·1)
	−	−	−	+	14	1·0					
	−	−	+	−	13	0·9					
	+	+	+	−	1	0·07					
	−	−	−	−	1297	89·3					
Total No reactive (%)	42 (2·9)	111 (7·6)	141 (9·7)	141 (9·7)							

+ Reactive (including borderline results) − non-reactive
D = diluted CSF U = undiluted CSF

TABLE II *Comparative results of the VDRL-CSF, MHA:TP-DCSF, MHA:TP-UCSF and FTA-CSF tests on samples of CSF from patients with diseases other than syphilis*

	Tests						Tests				
Diseases	VDRL	MHA-D	MHA-U	FTA	Total No	Diseases	VDRL	MHA-D	MHA-U	FTA	Total No
Aetiology unknown	−	−	−	−	1	Myelitis	−	−	−	−	7
Anaemia	−	−	−	−	1	Neurone lesion	−	−	−	−	3
Ataxia	−	−	−	−	1	Neurone lesion	−	−	−	+	1
Cerebral bilateral lesion	−	−	−	−	1	Neuritis	−	−	−	−	9
Cerebral haemorrhage	−	−	−	−	4	Pituitary tumour	−	−	−	−	2
Cerebral syndrome	−	−	−	−	6	Pneumonia	−	−	−	−	1
Cerebral tumour	−	−	−	−	4	Polyneuropathy	−	−	−	−	3
Cerebrovascular accident	−	−	−	−	11	Posterior fossa tumour	−	−	−	−	2
Coma	−	−	−	−	5	Posterior liver tumour	−	−	−	−	1
Dementia	−	−	−	−	8	Pseudobulba	−	−	−	−	1
Demyelinisation	−	−	−	−	2	Psychosis	−	−	−	−	4
Encephalomyeloneuropathy	−	−	−	−	13	Sciatica	−	−	−	−	2
Epilepsy	−	−	−	−	7	Seizures	−	−	−	−	12
Hepatitis	−	−	−	−	4	Sensation absence	−	−	−	−	1
Hypertension	−	−	−	−	9	Spastic quadriplegia	−	−	−	−	3
Hyperthermia	−	−	−	−	3	Stroke	−	−	−	−	1
Hypophyseal tumour	−	−	−	−	3	Syringomyella	−	−	−	−	1
Infectious diseases	−	−	−	−	4	Retinal artery occlusion	−	−	−	−	1
Intramedullary tumour	−	−	−	−	1	Right C4 radiculopathy	−	−	−	−	1
Lumbar adherence	−	−	−	−	3	Unstable angina	−	−	−	−	1
Lumbar radiculopathy	−	−	−	+	1	Vasculitis	−	−	−	−	2
L5 root compression	−	−	−	−	4	Vertebral disc protrusion	−	−	−	−	40
L5 & S1 discopathy	−	−	−	−	1	Vertebral disc protrusion	−	−	−	1+	1
L5 & S1 spondylolisthesis	−	−	−	−	3	Visual defect	−	−	−	−	2
Lumbar spinal block	−	−	−	−	1	Wernicke Korsakoff's disease	−	−	−	−	2
Meningitis	−	−	−	−	25	Wernicke Korsakoff's disease	−	−	−	+	1
Multiple sclerosis	−	−	−	−	16						

+ Reactive − non-reactive

TABLE III *Comparative results of the VDRL-CSF, MHA:TP-DCSF, MHA:TP-UCSF and FTA-CSF tests on 210 CSF samples from known cases of syphilis*

		% Reactivity for tests				% Agreement between tests				
Syphilis	No of cases	VDRL	MHA-D	MHA-U	FTA	VDRL: MHA-D	VDRL: MHA-U	VDRL: FTA	MHA-D: FTA	MHA-U: FTA
Primary	17	00·0	00·0	00·0	00·0	100·0	100·0	100·0	100·0	100·0
Secondary	27	18·5	48·2	66·6	66·6	70·4	51·9	51·9	81·5	92·6
Tertiary	83	37·4	80·7	90·4	90·4	56·6	47·0	51·8	95·2	100·0
Latent	77	7·8	40·3	59·8	53·3	67·5	48·1	52·0	84·4	72·7
Congenital	6	00·0	00·0	33·3	50·0	66·7	100·0	50·0	83·3	50·0
Total	210	20·0	52·9	67·1	65·2					

D = diluted CSF U = undiluted CSF

The performance of the MHA:TP-UCSF test with undiluted CSF did not decrease its specificity, since the samples from 247 patients with neurological or other disorders did not show any reactivity to this test.

These data indicate that the MHA:TP-UCSF test—although further evaluation is necessary—is as specific and sensitive, if not more so, as the FTA-CSF test and can be used to determine the anti-treponemal antibodies in CSF, especially in late secondary and tertiary syphilis.

We would like to acknowledge the valuable help of Danielle Coutu for criticisms and presentation of the manuscript and secretarial assistance.

References

1. Rathlev T. Haemagglutination test utilizing pathogenic *Treponema pallidum* for the serodiagnosis of syphilis. *Br J Vener Dis* 1967; **43:** 181-5.
2. Birry A, Kasatiya S. The evaluation of microhaemagglutination assay to determine the treponemal antibodies in cerebrospinal fluid (MHA:TP-CSF). *Br J Vener Dis* 1979; **55:** 239-44.
3. Jaffe HW, Larsen SA, Peters M, Jove DF, Lopez B, Schroeter AL. Tests for treponemal antibodies in CSF. *Arch Int Med* 1978; **138:** 252-5.
4. Monsiorska-Borowska L. TPHA reaction in cerebrospinal fluid in late syphilis. *Przegl Dermatol* 1977; **64:** 705-10.
5. Tomizawa T, Kasamatsu S, Yamaya S. Usefulness of the hemagglutination test using *Treponema pallidum* antigen (TPHA) for the serodiagnosis of syphilis. *Jpn J Med Sci Biol* 1969; **22:** 341-50.
6. National Communicable Disease Center, US Department of Health, Education and Welfare (ed). Microhemagglutination assay for *Treponema pallidum* antibodies (MHA-TP). USPHS publication. Washington DC: US Government Printing Office, 1969.
7. Kasatiya SS, Caloenescu M, Birry A. Automated fluorescent treponemal antibody (AFTA) test in cerebrospinal fluids in the diagnosis of neurosyphilis. *Can J Public Health* 1972; **63:** 447-52.
8. Leclerc G, Giroux M, Birry A, Kasatiya S. Study of fluorescent treponemal antibody test on cerebrospinal fluid using monospecific anti-immunoglobulin conjugates IgG, IgM and IgA. *Br J Vener Dis* 1978; **54:** 303-8.
9. National Communicable Disease Center, US Department of Health, Education and Welfare (ed). *Manual of Serological Tests.* USPH publication No 411. Washington DC: US Government Printing Office, 1969.
10. Duncan WP, Jenkins TW, Parham CE. Fluorescent treponemal antibody-cerebrospinal fluid (FTA-CSF) test. A provisional technique. *Br J Vener Dis* 1972; **48:** 97-101.
11. Kasatiya SS, Birry A. The use of incident light in immunofluorescence applied to syphilis serology. *Amer J Clin Pathol* 1972; **57:** 395-9.
12. Jephcott AE, Beveridge MAM, Sequiera PJL. Early TPHA response in primary syphilis. *Br J Vener Dis* 1977; **58:** 147.

Br J Vener Dis 1980;**56:**81-2.

Assessment of the Till-U-Test GC slide

W H DONALD
From the Department of Genitourinary Medicine, Derbyshire Royal Infirmary, Derby

SUMMARY The Till-U-Test (TUT) GC slide, a simple cultural procedure for *Neisseria gonorrhoeae,* was compared with a standard procedure using modified Thayer-Martin medium for laboratory confirmation of gonorrhoea in 144 patients. The TUT proved to be slightly more sensitive than the standard procedure. In 92·6% of all patients the results agreed, and in cases with positive results to Gram stain *N gonorrhoeae* was isolated in 93·6% with the TUT compared with 88·9% with the standard procedure. The TUT slide was easy to handle and produced results sooner than conventional methods. Thus the TUT could be very useful for presumptive diagnosis of gonorrhoea when full laboratory facilities are not readily available, as very little equipment is required.

Introduction

Numerous methods for laboratory diagnosis of gonorrhoea are available, including simplified tests such as the Microcult GC.[1] This study was undertaken to assess the recently developed cultural procedure, the Till-U-Test (TUT) GC slide (Tillomed Laboratories), for the growth of *Neisseria gonorrhoeae* from a direct inoculum on a Thayer-Martin medium with colistin replacing polymixin. Transport media are unnecessary, as the inoculation can be made with a swab or loop of material from the patient and the slide placed immediately in its own CO_2 environment. The inoculated slide may then be incubated in the clinic or sent to the laboratory for incubation and full identification. The slide consists of a sterile tongue covered on both sides with a brown medium. The tongue is fitted to a cap which screws into a sterile container, inside which a CO_2 atmosphere can be generated. The TUT was assessed in comparison with an established selective cultural method for its ability to detect *N gonorrhoeae* in clinical specimens containing the organism.

Patients and methods

The TUT was used in 67 male patients being investigated for urethritis and in 77 female patients presenting with genitourinary symptoms or as contacts of a male patient with gonorrhoea or non-specific urethritis. A total of 221 specimens were examined. In all cases Gram-stained smears were examined for Gram-negative intracellular diplococci.

Specimens for Gram-staining were taken from each patient with a sterile platinum loop. Specimens for culture were taken with a sterile swab; after inoculation of the TUT the swab was placed in Amies's transport medium. The TUT was inoculated according to the manufacturers' instructions by rubbing the swab gently over the surface of both sides of the slide; before the slide was replaced in the container the contents of a CO_2 generating capsule were tipped on to a moist sponge at the bottom of the container and the tongue replaced. The date and time of inoculation were entered on the label. The container was then transferred immediately to an incubator. The TUT container and the swab in Amies's transport medium were both taken to the microbiological laboratory between two and 18 hours after inoculation.

In the laboratory incubation of the TUT continued at 37°C. The swab was inoculated on to a non-selective medium (ordinary blood agar plate) and on to a selective medium, Thayer-Martin medium with the addition of trimethoprim and a reduction in concentration of vancomycin (1500 μg/ml) and polymixin (20 000 IU/ml). Cultures were examined 48 hours after inoculation and suspect colonies tested with oxidase reagent. Oxidase-positive colonies were subcultured for fermentation reactions and a specimen from the colony was Gram-stained to confirm the presence of Gram-negative diplococci. *N gonorrhoeae* were identified as Gram-negative diplococci with typical-looking oxidase-positive colonies which fermented glucose only.

Address for reprints: Dr W H Donald, Department of Genitourinary Medicine, Derbyshire Royal Infirmary, Derby

Received for publication 11 August 1979

Results

There was a close correlation between the findings of the two cultural methods; slightly more positive results were obtained with the TUT than with the standard cultural procedure (Table I). In 133 (92·3%) of the 144 patients the results agreed, and, of the 63 cases with typical-looking organisms on Gram-stain, positive results were obtained with the TUT in 59 (93·6%) compared with 56 (88·9%) with standard cultures. Of the 81 cases showing no organisms on Gram-stained smear, positive results were obtained with the TUT in three (3·7%) cases and with standard cultures in two (2·5%) (Table II). The detection rate for *N gonorrhoeae* from all specimens was high, particularly from cervical swabs (Table II).

TABLE I *Results of Till-U-Test compared with those using standard cultures from 144 patients*

Method	*Results*							
Gram-stain	+	+	+	+	−	−	−	−
TUT	+	+	−	−	+	+	−	−
Standard culture	+	−	+	−	+	−	+	−
No of patients	53	6	3	1	2	1	0	78

+ Positive for *N gonorrhoeae* − negative

TABLE II *Isolation of N gonorrhoeae from total specimens*

	Men		*Women*			
	Urethra		*Urethra*		*Cervix*	
Gram stain	+	−	+	−	+	−
Total specimens	31	36	22	55	26	51
TUT	28	2	19	8	25	5
Standard culture	26	2	18	6	25	4

+ Positive for *N gonorrhoeae* − negative

Discussion

A Gram stain of urethral discharge in men has been regarded as 99% accurate in the diagnosis of gonorrhoea if positive results are obtained,[2] but gonococci may be recovered by culture in >5% of cases with negative results[3] which were diagnosed initially as non-specific urethritis. Cultural diagnosis is therefore desirable in cases with negative results to Gram stain. In women, Gram stains are much less reliable, and cultures are therefore essential for a satisfactory diagnosis.

The principle of the TUT is a presumptive diagnosis of gonorrhoea by means of growth on a selective medium of typical colonial characteristics, oxidase reaction, and Gram stain. The test would not distinguish other *Neisseria* so it could not be reliably used for pharyngeal specimens, but when such facilities were available subculturing for sugar fermentation for further identification could be carried out.

The TUT stores satisfactorily for a minimum of three months at room temperature and requires only sterile swabs, Gram-staining equipment, a microscope, and an incubator. As growth was often evident after 24 hours' incubation, a combination of TUT and immunofluorescent staining could provide a report within 24 to 36 hours.

A great advantage of the TUT is that it can be incubated directly in its own CO_2 atmosphere, so that transfer problems associated with conventional methods do not arise. The TUT could be a valuable diagnostic aid where full laboratory facilities are not immediately available.

I thank Mrs Kathryn Boddy, who supervised and reported on the Till-U-Test GC slides in the Microbiology Laboratory, and Mr L Glick of Tillomed Laboratories Ltd for kindly supplying the Till-U-Test GC slides.

References

1. Willcox RR, John J. Simplified method for the cultural diagnosis of gonorrhoea. *Br J Vener Dis* 1976; **52:** 256.
2. Flumara NS. The diagnosis and treatment of gonorrhoea. *Med Clin North Am* 1972; **56:** 1105-14.
3. Willcox RR, Sparrow RW, Fazluddin GM. Minocycline in the treatment of non-gonococcal urethritis. *Br J Vener Dis* 1975; **51:** 206-9.

Br J Vener Dis 1980;**56**:83-7.

Delay and inhibition of growth of Neisseria gonorrhoeae in vitro

Relationship of delayed incubation, penicillinase production, urine culture, and self-treatment

WILLIAM G WESTBROOK III

From the Microbiology Laboratory, USAF Hospital, Clark Air Base, Republic of the Philippines

SUMMARY Delay in the appearance of *Neisseria gonorrhoeae* on Thayer-Martin medium was associated with delay in incubation and with the use of urine samples from infected men for cultural diagnosis. Self-treatment among women was related to inhibition of growth of *N gonorrhoeae,* particularly of non-penicillinase-producing gonococci (β-lactamase). The difference in the time of appearance of penicillinase-producing and non-penicillinase-producing gonococci was significant in men but not in women.

Introduction

The laboratory plays a vital role in the diagnosis of most sexually transmitted diseases, but this role is particularly essential in gonorrhoea. The studies reported here were the result of a need to expand and refine laboratory techniques to diagnose gonorrhoea in a military and a local civilian population at Clark Air Base, Republic of Philippines. Gonorrhoea has been highly prevalent for years in this mobile and sexually promiscuous population, but the usual public health measures kept the infection under reasonable control until penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strains emerged in the summer of 1976.[1 2]

Because PPNG strains could be identified only after cultural isolation, the bacteriological laboratory had to increase its workload several-fold to examine diagnostic and test-of-cure samples from the treatment clinic, gonorrhoea screening specimens from military and dependent women, and routine surveillance samples from local "hostesses" attending two local city public health clinics. Incubation of cultures from these clinics, located several miles away from the bacteriological laboratory, was delayed for one to four hours. It was necessary to determine the effect of delayed incubation of inoculated cultures on the recovery of both penicillinase-producing and non-penicillinase-producing *N gonorrhoeae* (non-PPNG).

An attempt was also made to increase surveillance for gonorrhoea in men, including those with no symptoms or signs of infection who could be contributing to the spread of gonorrhoea in this community.[3] Because of real or imagined trauma caused by collection of urethral specimens with a wire loop or a calcium-alginate swab, men were reluctant to volunteer for urethral culture when they were asymptomatic. We decided to compare the sensitivity of unspun urine culture with the standard urethral culture method in men with and without symptoms of gonorrhoea with a view to substituting urine for urethral culture as has been reported.[4]

A final consideration was the effect of self-treatment on the recovery of *N gonorrhoeae* by culture. Self-treatment was acknowledged to be common among women attending the city clinics and was thought to be contributing to the selection and spread of penicillin-resistant gonococci.

Patients and methods

Modified Thayer-Martin (MTM) medium was used for culture of *N gonorrhoeae* throughout the study.[5] Each batch of medium was prepared and tested locally for its ability to support the growth of gonococci.

The studies were carried out with the co-operation of: (a) men attending a military hospital clinic; (b) women attending a military hospital gynaecological clinic; (c) women attending a local city health clinic; and (d) local civilian men.

Address for reprints: Captain W G Westbrook, Clinical Laboratory, USAF Regional Hospital, MacDill Air Force Base, Florida 33608, USA

Received for publication 6 April 1979

COLLECTION OF SPECIMENS

Urethral specimens from men were obtained using calcium-alginate swabs. Endocervical culture specimens were collected with cotton swabs. Urethral specimens for culture were obtained from all men and endocervical specimens from all women. Urine specimens from selected men and women were obtained for culture and other tests. The aim was to secure the first 10 ml of urine voided in the morning or after an interval of four hours since the last voiding. Urine was collected in a sterile plastic container and cultured within one minute. A sterile cotton swab was swirled in the urine to entrap bacteria and excess urine eliminated by rolling the swab against the wall of the container before inoculating the cultures. Swabbing in a "Z" pattern across the culture medium was routine.

CULTURAL TECHNIQUES

All MTM plate inoculations took place within one minute of securing samples. Plates were placed in a CO_2 candle jar within five minutes. Incubation at 36 ± 1°C took place within one hour in the case of men; within two hours in the case of women attending the gynaecological clinic; and within five hours in the case of women attending local health clinics.

IDENTIFICATION

All MTM plates were examined every 18-24 hours for a total of 96 hours. When typical gonococcal colonies were first seen, the incubation time was recorded. Typical colonies were presumptively identified as *N gonorrhoeae* by oxidase reaction and examination of Gram-stained smears of suspected colonies. Owing to the large workload, presumed gonococci were not submitted to confirmatory tests. Penicillin sensitivity was determined by the standardised disc-diffusion susceptibility test (10^8 colony-forming units per ml) on enriched chocolate agar (less than 20 mm diameter zone of inhibition around a disc containing 10µg penicillin indicating resistance).[6] Penicillinase production was detected by the chromogenic cephalosporin test.[7] Positive and negative controls were used with all tests.

BIOASSAY TECHNIQUE

Mid-stream urine specimens from 512 consecutive women were examined for the presence of urinary bacterial inhibitory substances (UBIS). One drop of fresh urine from each of 12 patients was placed on the surface of a 150-mm diameter bioassay plate. The bioassay plates contained Muller-Hinton agar seeded with *Sarcina lutea (Micrococcus luteus)* FDA strain 1001, susceptible to $\leq 0{\cdot}01$ µg/ml of penicillin. All urine specimens tested were stored at −10°C for possible retesting. Bioassay plates were capable of detecting UBIS in urine from two healthy men between one and eight hours after ingestion of 250 mg of ampicillin by inhibiting growth of *S lutea*. UBIS was also detected in urine in these men 24 hours after taking 500 mg of ampicillin by mouth.

Inhibition zones on bioassay plates were measured after 18-24 hours' incubation. When zones of inhibition coalesced or otherwise proved difficult to read, testing was repeated on plates allowing large areas for inhibition. Aliquots (0·2 ml) of all urine specimens found to contain UBIS were mixed with an equal volume of commerically obtained penicillinase concentrate (10×10^6 kinetic units per ml) (Baltimore Biologicals, Cockeysville, Maryland) and incubated for 30 minutes at room temperature before being retested.

STATISTICAL ANALYSIS

Mean differences between the incubation times of the different groups cultured were tested by Student's *t* test, assuming unequal variances. χ^2 analysis was used to compare (a) the mean difference between the incubation times of the different groups; (b) the growth of *N gonorrhoeae* at 24-hour intervals in the different populations studied; (c) the time of incubation needed to isolate *N gonorrhoeae* from urine and urethral specimens when both were culture-positive in the same patient and whether or not symptoms of gonorrhoea were present; and (d) the effect of self-treatment on the recovery of *N gonorrhoeae.*

Results

DELAYED INCUBATION

Of 63 962 specimens examined, 5254 (8·2%) gave a positive result for *N gonorrhoeae* according to the criteria. The mean time to appearance of colonies was directly related to the time-lapse between inoculation of MTM plates and incubation at 36±1°C. Significantly longer incubation was needed to detect growth of gonococci isolated from women attending city clinics than from women attending the hospital gynaecological clinic and men attending the hospital outpatient clinic (Table I). A significantly longer time of incubation was needed for the isolation of PPNG in men than for non-PPNG; this was not true in either group of women.

PENICILLINASE-PRODUCING GONOCOCCI

Of the 1801 positive cultures from men, 304 (21·9%) grew PPNG strains. Of 3453 positive cultures from women, 683 (19·8%) grew PPNG strains. In men 96·9% of isolates were identified within 48 hours of incubation whereas only 81·8% of the women attending the hospital gynaecological clinic had identifiable colonies at 48 hours (Table II). The figure for women attending the local health clinic was

TABLE I *Mean incubation time for growth and isolation of gonococci*

Clinic	*Type of gonococcus*	*Maximum delay before incubation*	*Mean incubation time (±SD) of positive cultures*	*Significance**	*Probability*
(1) City (F)	PPNG + non-PPNG	5 hr	54·9±15·2 hrs	(1) *vs* (2) $t = 5\cdot5$	<0·01
(2) Hospital (F)	PPNG + non-PPNG	2 hr	45·6±17·5 hrs	(2) *vs* (3) $t = 7\cdot1$	<0·01
(3) Hospital (M)	PPNG	1 hr	32·8±13·5 hrs	(3) *vs* (4) $t = 2\cdot2$	<0·05
(4) Hospital (M)	non-PPNG	1 hr	31·2±13·0 hrs	(2) *vs* (4) $t = 8\cdot45$	<0·01

*Student's *t* test

TABLE II *Time of incubation for cultures of N gonorrhoeae*

Clinic	*Total No*	*No of PPNG/total positive cultures (cumulative %) after*				*Total*
		24 hr	*48 hr*	*72 hr*	*96 hr*	
Hospital (M)	8612	264/1301 (72·2)	116/444 (96·9)	13/47 (99·5)	1/9 (100)	394/1801
Hospital (F)	6863	8/33 (30·0)	10/57 (81·8)	1/18 (98·2)	1/2 (100)	20/110
City (F)	48487	19/107 (3·2)	476/2385 (74·5)	134/631 (93·4)	34/220 (100)	663/3343
Total	63962	291/1441 (27·4)	602/2886 (82·3)	148/801 (97·5)	36/231 (100)	1077/5254

PPNG = penicillinase-producing *N gonorrhoeae*

74·5%. The differences in time of incubation required to detect growth of *N gonorrhoeae* in each of these groups was highly significant at 48 hours ($\chi^2_2 = 458\cdot5$, $P < 0\cdot001$) and significant at 72 hours ($\chi^2_2 = 6\cdot2$, $P < 0\cdot05$) but not before 48 hours ($\chi^2_2 = 1\cdot15$) or after 72 hours ($\chi^2_2 = 2\cdot48$).

URINE AND URETHRAL CULTURES (MEN)

All but 64 of the 985 paired urethral and urine samples from men were collected before the first urine of the day was passed or four hours or more after the last voiding.

Two hundred and eighty-four urethral specimens and 279 urine specimens gave positive results, that is a 98·2% agreement. Urine sampling was associated with a significant delay in the appearance of gonococcal colonies ($\chi^2_3 = 38\cdot59$, $P < 0\cdot001$) and of PPNG ($\chi^2_1 = 8\cdot09$, $P < 0\cdot02$) compared with urethral sampling (Table III). This also applied to the 141 infected men whose symptoms were known irrespective of whether or not the organisms were PPNG strains (Table IV).

A total of 115 men with no urethral discharge or complaints of dysuria had both urine and urethral cultures performed. Seven (6·1%) of these men were infected with *N gonorrhoeae,* which was detected by both urine and urethral culture.

Of a separate group of 111 local civilian men who had urine cultures performed later, five yielded non-PPNG isolates. Three of the five men denied having symptoms of gonorrhoea.

TABLE III *Number of N gonorrhoeae isolates identified at 24-hour intervals in the 279 cases for which both urine and urethral cultures gave positive results*

Incubation time (hrs)	*No culture-positive (PPNG)*	
	Urine	*Urethra*
24	163 (34)	229 (48)
48	103 (15)	47 (7)
72	11 (4)	3
96	2 (2)	0
Total	279 (55)	279 (55)

PPNG = penicillinase-producing *N gonorrhoeae*

TABLE IV *Number of N gonorrhoeae isolates identified at 24-hour intervals in 141 cases for which both urine and urethral cultures gave positive results and symptoms were known*

	Incubation (hrs)	*No culture-positive (PPNG)*	
		Urine	*Urethra*
Symptomatic (n = 134)	24	90 (21)*	116 (24)
	48	40 (5)	17 (4)
	72	4 (2)	1
Asymptomatic (n = 7)	24	3	6 (1)
	48	4 (1)	1
	72	0	0
Total		141 (29)	141 (29)

*$\chi^2_2 = 11\cdot49$, $P < 0\cdot01$
PPNG = penicillinase-producing *N gonorrhoeae*

SELF-TREATMENT

Of 591 urine samples from female patients, 93 (15·7%) contained UBIS (Table V). When the 93 samples were reacted with penicillinase concentrate, 73 failed to show UBIS suggesting that they had originally contained a β-lactam antibiotic-like penicillin or one of the cephalosporins. Two of these patients had been prescribed ampicillin by a physician within the previous 96 hours. The 20 patients with repeat positive results to the UBIS tests were considered to have taken antibiotics other than those mentioned.

Only two (2·2%) of the 93 patients with UBIS had positive endocervical culture results whereas 36 (7·2%) of 498 without evidence of self-treatment had positive results, a difference which was significant ($\chi^2_1 = 6{\cdot}133$, P<0·05). Of the two patients with UBIS who had positive culture results, both had PPNG strains. Of the 36 isolates from UBIS-negative patients, six (16·6%) were PPNG.

Discussion

The significant delay in recovering *N gonorrhoeae* from women by culture beyond the usual 48 hours of incubation could be due to one or several of the following factors: (a) the delay in processing inoculated cultures before incubation at 36±1°C; (b) the bacteriostatic activity of antimicrobial substances in the endocervical and vaginal secretions; (c) a smaller inoculum of gonococci from the endocervix compared with that from the urethra in men; and (d) a difference in the growth characteristics of gonococci isolated from women. A delay of two or more hours before incubation under CO_2 at 36°C can produce a significant delay in the appearance of gonococcal colonies and a reduction in their size and number.[8] This is the most logical explanation for the delay in recovering gonococci from women by culture in this study. Changes were made to ensure more prompt processing of cultures from female clinics. Even under the most ideal conditions, however, a small number of both PPNG and non-PPNG strains required more than 48 hours of incubation before growth could be detected.

TABLE V *Comparison of urine specimens tested for growth inhibitory substances and isolation of N gonorrhoeae by endocervical Thayer-Martin culture*

		No culture-positive	
Urine specimens	*Culture*	*Non-PPNG (%)*	*PPNG (%)*
Non-inhibitory	498	30 (6·0)	6 (1·2)
Inhibitory*	93	0†	2 (2·2)

*Containing penicillin-like (73) or other (20) inhibitory substances
†$\chi^2_1 = 6{\cdot}133$, P<0·05

The culture of unspun urine gave results comparable to culture of urethral specimens obtained directly by swab in men with symptomatic and asymptomatic infections. The significantly longer incubation time needed to isolate *N gonorrhoeae* from urine was probably due to the smaller number of gonococci contained in the urine. The collection of urine specimens for culture was more acceptable to patients than was that of urethral specimens; because of this the culture of the former might be more appropriate for screening large numbers of asymptomatically infected men. The need for prolonged incubation of urine cultures might be overcome by adding a small volume of concentrated MTM medium (minus agar) to the urine in the collection jar. Pre-incubation of this mixture might allow gonococci to multiply in the urine, thus increasing inoculum size.

The finding of penicillin-like substances in the urine of 12·4% of women attending the local city health clinic indicates the use of oral antibiotics for self-treatment or for prophylaxis of gonorrhoea in this population. Possibly the practice contributes to the selection and dissemination of drug-resistant strains of *N gonorrhoeae,* including PPNG. These women had been told repeatedly that self-medication was dangerous and should not be practised and that there was no need for it, since all diagnostic and therapeutic services at the clinic were provided free. Thus continued patient education is necessary as well as improvements in the diagnosis and treatment of gonorrhoea in the Philippines.

I would like to thank Staff Sergeant Charles J Slabisak, CLA (ASCP), Mrs Adeluisa C Prater, RMT, Miss Elaine L Corre, RMT, Mr Doroteo B Dingcol, RMT, Mrs Cynthia C Henson, MT (ASCP), Mr Romeo V Reyes Jr, MT, and Miss Violeta A Villapando, MT, without whose help this study would not have been possible. My thanks to Colonel Robert R Smith, Lieutenant Colonel Donald G Wright, and Ignacia R DeJesus, for their co-operation, to Dr R S Morton and Mr Akbar Zaidi for their assistance in preparing the manuscript, and, especially, to Peter L Perine, for his advice and close support.

Sarcina lutea, FDA strain 1001, was kindly supplied by J S Lewis, Center for Disease Control, Atlanta, Georgia, USA.

References

1. Ashford WA, Golash RG, Hemming VG. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976; **2:** 657-8.
2. Phillips I. β-lactamase-producing penicillin-resistant gonococcus. *Lancet* 1976; **2:** 656-7.

3. Handsfield, HH, Lipman TO, Harnisch JP, Tronca E, Holmes KK. Asymptomatic gonorrhea in men. *N Engl J Med* 1974; **290**: 117-23.
4. Feng WC, Medeiros AA, Murray ES. Diagnosis of gonorrhea in male patients by culture of uncentrifuged first-voided urine. *JAMA* 1977; **237**: 896-7.
5. Thayer JD, Martin JE jun. An improved medium selective for cultivation of gonorrhoeae and meningitidis. *Public Health Rep* 1966; **81**: 559-62.
6. Biddle JW, Swenson JM, Thornsberry S. Disc agar diffusion antimicrobial susceptibility tests with β-lactamase producing *Neisseria gonorrhoeae*. *J Antibiot* 1978; **31**: 352-8.
7. O'Callaghan CH, Morris A, Kirby SM, Shingler AH. Novel method for detection of β-lactamases by using a chromogenic cephalosporin substrate. *Antimicrob Agents Chemother* 1972; **1**: 283-8.
8. Chapel TA, Smeltzer M, Dassel R. The effect of delaying incubation in a CO_2-enriched environment on gonococci. *Health Lab Sci Rep* 1976; **13**: 45-8.

Br J Vener Dis 1980;56:88-91.

Prolonged use of the Greenland method of treatment of gonorrhoea

A J EVANS,* G D MORRISON,* AND D J PRICE†

*From the *Department of Venereology, Plymouth General Hospital, and the †Department of Biological Sciences, Plymouth Polytechnic, Plymouth, Devon*

SUMMARY Of 1081 patients with gonorrhoea treated with probenecid 1 g orally and benzyl penicillin 5 megaunits intramuscularly, 96·7% were cured and no complications were encountered. This treatment regimen was associated with a pronounced reduction in the proportion of gonococcal strains showing diminished sensitivity to penicillin, from 51% to between 4% and 14% over a period of four years. When this treatment method was abandoned, however, the percentage of less sensitive strains rose to 33% over the following 18 months.

Introduction

By 1971 we were becoming seriously worried because 43% of gonococci isolated in Plymouth showed diminished sensitivity to penicillin in vitro (a zone of inhibition corresponding to a minimum inhibitory concentration (MIC) $<0\cdot1\mu g/ml$). In 1969 and 1970 our routine treatment for uncomplicated gonorrhoea in men and women had been with co-trimoxazole tablets *BP* (4 tablets initially and then 2 tablets twice daily for five days).

Olsen and Lomholt[1] reported in 1969 from Greenland that when probenecid 1 g and benzyl penicillin 5 megaunits had been introduced as the routine treatment for gonorrhoea the proportion of strains less sensitive to penicillin had fallen noticeably and had remained low. Gray *et al*[2] suggested that the use of this regimen nationally might result in a substantial reduction in the prevalence of less sensitive strains.

In the hope of achieving a similar reduction in these less sensitive strains we adopted Olsen's and Lomholt's[1] method of treatment, which we have called the Greenland method. From February 1971 to May 1977 this was the standard treatment for gonorrhoea in the clinic at Plymouth. For the next six months alternate cases were treated with either cefuroxime (1 g i.m. and 1 g probenecid orally) or the Greenland method. Throughout 1978 all cases were treated with cefuroxime (either 1·5 g i.m. or 0·75 g plus 1 g probenecid).

The results with this regimen, particularly the changes in the sensitivity pattern of the gonococci isolated, are reported here.

Patients and methods

STUDY POPULATION

Between February 1971 and May 1977, 1925 cases of gonorrhoea (1227 men and 698 women) were treated with the Greenland method. Only patients who were hypersensitive to penicillin, those with disseminated gonococcal infection or salpingitis, and the rare patient who refused injection were excluded from the series.

The age and racial distribution of the patients remained the same throughout each year of the series. The only discernible change over the period was that the male-to-female ratio changed from 1·7 : 1 to 1·4 : 1.

Of the initial 1925 patients, 844 (632 men and 212 women) were withdrawn from the series or else defaulted or were transferred elsewhere without follow up. The chief reason for withdrawal was because the patient had been given tetracyline for post-gonococcal urethritis during the follow-up period. As a result the withdrawal/default rate is much higher in men (51%) than in women (30%). There remained therefore 1081 cases (595 men and 486 women) for assessment.

DIAGNOSIS

Gonorrhoea was diagnosed on the finding of intracellular Gram-negative reniform diplococci in stained smears, or on the isolation of *Neisseria gonorrhoeae* on culture, or on both. Of the 1925 initial cases, gonococci were found in both Gram-

Address for reprints: Dr A J Evans, Treatment Clinic, Plymouth General Hospital, Freedom Fields, Plymouth, Devon

Received for publication 10 September 1979

stained smears and cultures in 1539 (80%)—that is, in 84% of men and 74% of women. In 297 (15%) cases the diagnosis was made on the basis of positive results to Gram-stained smears only (190 men and 107 women) and in 89 (5%) cases by culture only—13 (1%) men and 76 (11%) women.

Specimens for Gram-stain and culture were taken from the urethra in men and from the urethra and cervix in women and from the rectum in all male homosexuals. Throat swabs for culture were taken from all patients who admitted to orogenital contact, from all male homosexuals, and from any patient who was a known contact of gonorrhoea but from whom we had failed to isolate gonococci from other sites.

CULTURE AND SENSITIVITY TESTING

Culture and sensitivity testing methods and the criteria for distinguishing sensitive from less sensitive strains were as previously reported.[3] To ensure that these criteria had not changed during the period of this report, the MIC of benzyl penicillin (determined by plate diffusion) for 12 strains was compared with the zone of inhibition around a 1·5 unit disc (Oxoid). No change was shown to have occurred.

TREATMENT

All patients with gonorrhoea were treated with probenecid 1 g by mouth followed after about 15 minutes by a single intramuscular injection of benzyl penicillin 5 megaunits dissolved in 6·5 ml of 0·5% xylocaine to make a final volume of 8 ml (the Greenland method).

Patients were instructed to refrain from sexual intercourse for two weeks after treatment.

FOLLOW UP

Patients were seen at seven and 14 days after treatment. If any urethral discharge was found at either follow-up attendance in men who had had gonococcal urethritis, urethral smears and cultures were made. If no discharge was present, a two-glass urine specimen was examined and a smear made of any threads in the urine. At each attendance, specimens for Gram stain and culture were taken from the urethra and cervix in women, from the rectum in patients with gonococcal proctitis, and from the throat in patients with gonococcal pharyngitis.

If gonococci were found at any time within two weeks of treatment, and the patient denied further sexual intercourse, treatment was then considered to have failed. If gonococci were found after two weeks then reinfection was assumed to have occurred. No method of assessing which cases relapsed and which were reinfected is infallible; the method used here was suggested 20 years ago by Dallas.[4]

Results

Of the 1081 cases (595 men and 486 women) treated with the Greenland method and followed up at seven and 14 days, gonococci were isolated on culture during the follow-up period from 34 (20 men and 14 women) and two more had positive results only on Gram stain, making 36 failures in all. The cure rate was 96·7% (men 96·5%, women 97%). Of the men, 27 had gonococcal proctitis and two of them were not cured. Five patients had gonococcal pharyngitis and treatment failure occurred in one.

The failure rate was significantly higher ($\chi^2_1 = 5{\cdot}878$, $P<0{\cdot}02$) in patients infected with strains of gonococci with reduced sensitivity to penicillin. Of 919 cases followed up, and from whom strains of gonococci which were fully sensitive to penicillin were isolated, 25 were considered to be treatment failures, giving a failure rate of 2·7%, whereas of 162 cases followed up who had strains showing reduced sensitivity to penicillin 11 were treatment failures, giving a failure rate of 6·8%.

TABLE *Penicillin sensitivity of gonococci isolated between 1971 and 1977*

	Men		*Women*		*Total*	
Sensitivity to penicillin	*No*	*%*	*No*	*%*	*No*	*%*
Fully sensitive	831	84	490	87	1321	85
Diminished	162	16	74	13	236	15
Total	993		564		1557	

ANTIBIOTIC SENSITIVITIES

In 1628 cases gonococci were cultured from at least one site. In 71 cases the isolate died on subculture and antibiotic sensitivity could not therefore be assessed. Results of sensitivity testing to penicillin in the remaining 1557 cases (993 men and 564 women) are given in the Table. This shows that during the six years when the Greenland method was the routine treatment for gonorrhoea 15% of the isolates showed diminished sensitivity to penicillin. No β-lactamase-producing strains were found.

Since 1969 we have followed the variations in the percentage of strains of gonococci which showed diminished sensitivity to penicillin. These variations are shown for each six-month period in the Figure, together with the 95% confidence limits for a proportion calculated with a correction for continuity thus:

$$\hat{p} \mp \{1{\cdot}96\sqrt{(\hat{p}\hat{q}/n)} + \tfrac{1}{2}n\}$$

There is a highly significant difference ($\chi^2_2 = 67{\cdot}734$, $P<0{\cdot}001$) in the proportion of resistant strains between the three different treatment methods.

From mid-1969 to the beginning of 1971, while co-trimoxazole was the routine treatment, the percentage of less sensitive strains rose from 34 to 43%.

However the difference between the six-month periods is not significant ($\chi^2_1 = 1{\cdot}386$, $P = 0{\cdot}5$). For the first six-month period after the introduction of the Greenland method there was a further rise in the proportion of less sensitive strains to 51% (Figure); thereafter there was a rapid fall over the next two years to 5% in 1973. Use of the Greenland method was continued until mid-1977 and during that time the proportion remained low, within the limits of 3·5% to 12·4%. Thus there is a pronounced heterogeneity between these 13 samples, which is highly significant ($\chi^2_{12} = 197{\cdot}251$, $P < 0{\cdot}001$).

During the six-month period when alternate patients were treated by the Greenland method or with cefuroxime the proportion of less sensitive strains remained low (6%) (figure). During 1978, when cefuroxime alone was the routine treatment, the proportion of less sensitive strains rose to 25% and then to 33%. The difference between samples during this 18-month period is also significant ($\chi^2_2 = 36{\cdot}447$, $P < 0{\cdot}001$).

There has been one other change in the sensitivity pattern. Of all the strains of gonococci isolated in 1971 10% were resistant to streptomycin, and all the strains in that year which showed reduced sensitivity to penicillin were also resistant to streptomycin. Over the years the combination of reduced sensitivity to penicillin and resistance to streptomycin has become rare. Only two (0·8%) strains in 1978 showed resistance to streptomycin.

Discussion

The satisfactory results obtained with the Greenland method of treatment (a cure rate of 96·7%) is in keeping with the findings of the originators[1] of this

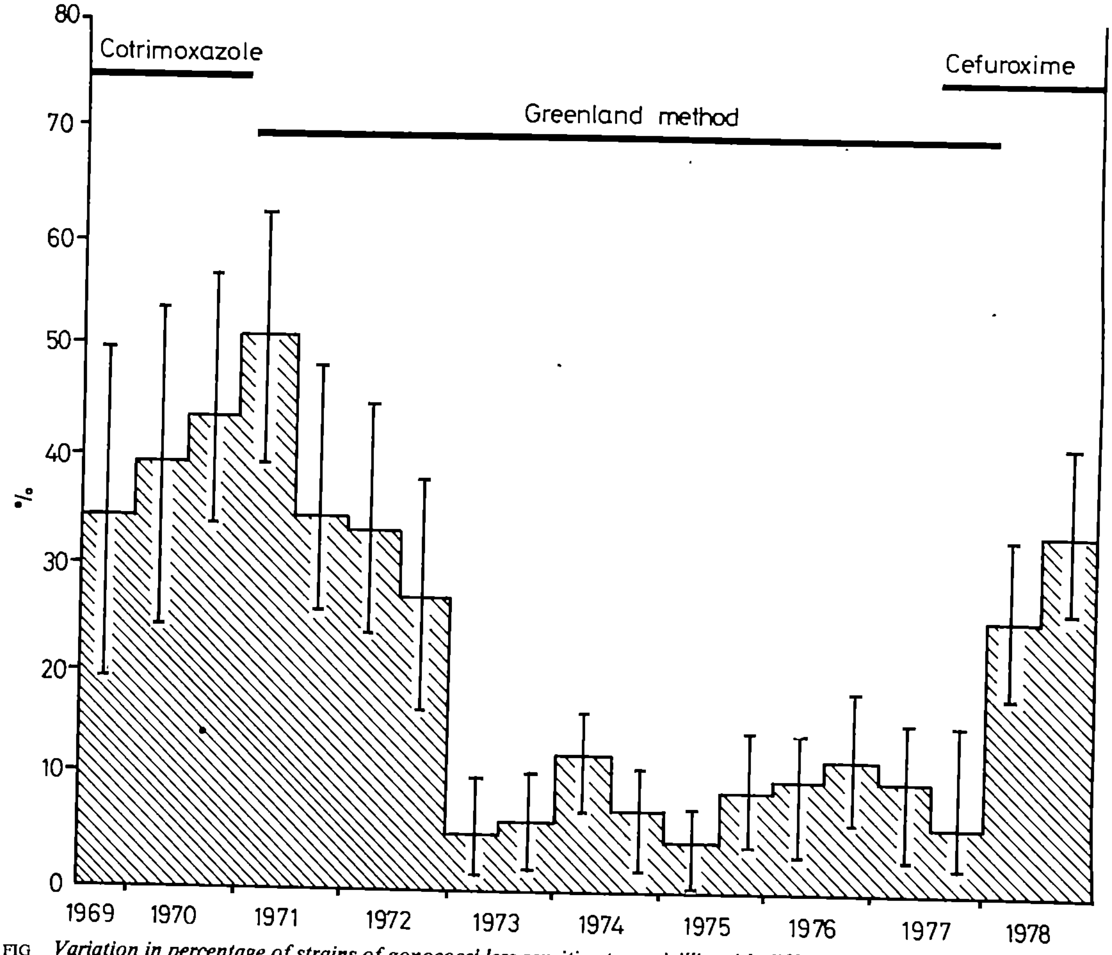


FIG *Variation in percentage of strains of gonococci less sensitive to penicillin with different treatment regimens (1969-78) and showing 95% confidence limits with correction for continuity* routine and of others.[2] Wigfield *et al*[5] suggested that the large volume (8ml) of the injection might be disadvantageous. This has not been our experience and we have not recorded any difficulties or major complications with its use.

Olsen and Lomholt[1] reported a pronounced reduction in Greenland in the proportion of strains of gonococci which were less sensitive to penicillin when their method of treatment was introduced. This improvement continued throughout the 3½ years covered by their report. Our results from Plymouth confirm these findings, although our series continued for longer (over six years).

What has not previously been reported is that on discontinuing treatment by the Greenland method the proportion of less sensitive strains rises sharply again.

It is difficult to see any reason for these changes. Wigfield *et al*[5] pointed out that the reduction in less sensitive strains could not be attributed to the efficiency of the method, since this would eliminate sensitive and less sensitive strains equally well, and so the proportion of less sensitive strains would remain the same.

There have been many reports of a wide variation in the prevalence of less sensitive strains in different centres, and at the same centres at different times.[6-8] The statistical analysis however shows that our results are most unlikely to be due to chance and that, whatever the reason, the use of the Greenland method of treatment is associated with a diminution of strains which are less sensitive to penicillin. We know of no other reports associating the therapeutic regimen with variation in the sensitivity pattern of the gonococci isolated.

The near disappearance of streptomycin-resistant strains is surprising. However, streptomycin is now never used for the treatment of gonorrhoea and this may explain the decrease in the number of streptomycin-resistant strains. A similar reduction in streptomycin-resistant strains has been reported from Denmark.[9]

Patients infected with strains of gonococci of reduced sensitivity to penicillin have a higher failure rate when treated with penicillin than do patients infected with fully sensitive strains.[6-8 10-12] Similar results have been obtained in the present series. Prolonged use of the Greenland method is associated with a reduction in less sensitive strains and should therefore result in a lower failure rate. This theoretical advantage is so great that we have now reverted to the use of the Greenland method as our standard treatment for gonorrhoea in Plymouth. It will be interesting to see if this is again associated with a reduction in the numbers of less sensitive strains of gonococci.

We are indebted to Dr Gillian M Churcher and her staff for carrying out the cultures and antibiotic sensitivities in her department. Our thanks are also due to Dr W H Haskins and to the nursing and secretarial staff of the clinic for their help in this study.

Addendum

Use of the Greenland method throughout 1979 has been associated with a fall in the proportion of strains less sensitive to penicillin from 33% to 22%.

References

1. Olsen GA, Lomholt G. Gonorrhoea treated by a combination of probenecid and sodium penicillin G. *Br J Vener Dis* 1969: **45:** 144-7.
2. Gray RCF, Phillips I, Nicol CS. Treatment of gonorrhoea with three different antibiotic regimens. *Br J Vener Dis* 1970: **46:** 401-3.
3. Evans AJ, Churcher GM, Human RP. Sulphamethoxazole-trimethoprim in the treatment of gonorrhoea. *Br J Vener Dis* 1972; **48:** 179-81.
4. Dallas NL. Penicillin treatment failures in male gonorrhoea. *Br J Vener Dis* 1958: **34:** 194-5.
5. Wigfield AS, Selkon JB, Rich GE. Single-session treatment of uncomplicated gonorrhoea using penicillin combined with cotrimoxazole. *Br J Vener Dis* 1973: **49:** 277-90.
6. Curtis FR, Wilkinson AE. Comparison of the in-vitro sensitivity of gonococci to penicillin with the results of treatment. *Br J Vener Dis* 1958: **34:** 70-82.
7. Medical Research Council Working Party. Resistance of gonococci to penicillin. *Lancet* 1961: **2:** 226-30.
8. Jaffe HW, Biddle JW, Thornberry C, *et al.* National gonorrhoea therapy monitoring study. In-vitro antibiotic susceptibility and its correlation with treatment results. *N Engl J Med* 1976: **294:** 5-9.
9. World Health Organisation. *Neisseria gonorrhoeae* and gonococcal infections. *WHO Tech Rep Ser* 1978: **616:** 81.
10. Craddock-Watson JE, Shotter PA, Nicol CS. Sensitivity of strains of gonococci to penicillin, sulphathiazole and streptomycin. *Br Med J* 1958: **1:** 1091-2.
11. Gjessing HC, Odegaard K. Intramuscular injection of procaine penicillin combined with oral administration of chloramphenicol in the treatment of gonorrhoea. *Br J Vener Dis* 1966: **42:** 107-9.
12. Evans AJ. Relapse of gonorrhoea after treatment with penicillin or streptomycin. *Br J Vener Dis* 1966: **42:** 251-62.

Br J Vener Dis 1980;**56**:92-5.

Value and feasibility of screening women attending STD clinics for cervical chlamydial infections

SHIRLEY J RICHMOND,* I D PAUL,* AND P K TAYLOR†

*From the *Public Health Laboratory, and the †Department of Venereology, Royal Infirmary, Bristol*

SUMMARY A chlamydial screening service was provided in Bristol over a three-month period for women attending the sexually transmitted disease (STD) clinic either for the first time or with a new complaint. Isolation of *Chlamydia trachomatis* was attempted in cytochalasin-treated McCoy cells. Of 919 specimens, valid results were obtained in 796. Chlamydial infections were identified and treated in 154 (19%) of these 796 women. Chlamydia were isolated from 52 (37%) of 152 female partners of men with nongonococcal urethritis (NGU); these patients already routinely receive treatment with tetracyclines in this clinic. The remaining 102 infections (34 (48%) of 71 women with gonorrhoea and 68 (12%) of the other 573 women) would have been unrecognised and usually untreated without chlamydial isolation studies. These figures confirm the need to provide chlamydial diagnostic services for selected STD clinic attenders.

Introduction

Patients attending sexually transmitted disease (STD) clinics are frequently infected with genital strains of *Chlamydia trachomatis*. These obligate intracellular bacterial parasites are sexually transmitted organisms, which are now recognised as an important cause of non-specific genital infections.[1-3] Definitive diagnosis of a genital chlamydial infection depends on isolation of the organism in in-vitro cell cultures, but since existing techniques are time-consuming and labour-intensive, it is difficult at present to provide routine screening of patients attending STD clinics on the scale now available for the diagnosis of infections with *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and *Candida albicans*.

Shedding of chlamydia from the male urethra is usually associated with the symptoms and signs of non-specific urethritis, which may present as either nongonococcal or post-gonococcal urethritis, for which the patient routinely receives treatment with tetracyclines. Chlamydia are sensitive to these antibiotics,[4 5] and therefore, whether or not laboratory facilities for the isolation of chlamydia are available, men with active urethral chlamydial infections usually receive appropriate antimicrobial therapy. In women, however, shedding of chlamydia from the cervix is not associated with distinct clinical features.[6-8] Moreover, apart from the female partners of men with nongonococcal urethritis (NGU) who are now frequently given epidemiological treatment on the basis of their partner's urethritis, women attending STD clinics do not routinely receive tetracyclines. Many cervical chlamydial infections therefore go both unrecognised and untreated.

In an attempt to identify these cervical infections, a chlamydial screening service was provided for all new female patients who presented at the STD clinic of the Bristol Royal Infirmary for a three-month period. The purpose of this study was to assess the feasibility of providing such a laboratory service and to establish the practical relevance of such a service to the clinician.

Patients and methods

STUDY GROUP

Patients consisted of women who attended the STD clinic at the Bristol Royal Infirmary for the first time between 19 February 1979 and 25 May 1979 and women who had attended the clinic previously but who presented during this period with a new complaint. Retrospectively patients were assigned to one of three categories: (a) those from whom *N gonorrhoeae* was isolated at the time they were screened for chlamydia; (b) those identified from their medical records as consorts of men with NGU; and (c) the remainder of the study group.

Address for reprints: Dr S J Richmond, Public Health Laboratory, Myrtle Road, Kingsdown, Bristol BS2 8EL

Received for publication 13 August 1979

SPECIMENS FOR CHLAMYDIAL ISOLATION

In addition to the specimens routinely collected from all patients for the diagnosis of infections with *N gonorrhoeae*, *T vaginalis*, and *C albicans*,[6] an endocervical swab for chlamydial isolation was obtained from each patient by a member of the nursing or medical staff and placed in sorbitol transport medium.[9] Each specimen was held at 4°C until transported to the laboratory where it was either inoculated into cell cultures or snap frozen at −70°C within 24 hours of being taken. Specimens were delivered to the laboratory each weekday morning and early afternoon, and on Friday evenings an additional delivery of specimens obtained at the evening clinic was made. Clinicians were asked to state whether or not the patient had received any antimicrobial therapy during the previous month.

CELL CULTURE TECHNIQUE

Isolation of *C trachomatis* was attempted in McCoy cells treated with cytochalasin B.[10] With this technique McCoy cells are treated with cytochalasin for three days before inoculation of the clinical specimen. Each Tuesday and Friday approximately 50 culture tubes (Nunc, Code 3-62707, Gibco-Biocult) containing a sterile circular glass coverslip 10 mm in diameter were each seeded with $1\cdot2 \times 10^5$ McCoy cells in 1 ml growth medium containing 1 μg cytochalasin and incubated at 35°C for three days. Cultures made on Friday were either inoculated on Monday or held at room temperature for 24 or 48 hours for inoculation of specimens received on Tuesdays and Wednesdays. Cultures made on Tuesday were inoculated on Friday. Cultures inoculated without being held at room temperature for 24 or 48 hours are subsequently referred to as "fresh" cultures. All specimens which arrived when cell cultures were available were inoculated within 24 hours of being taken but specimens received at times when cultures were not available were snap frozen and stored at −70°C until inoculation.

Each specimen was inoculated into one cell culture (0·25 ml inoculum) and then stored at −70°C until an unequivocal result was obtained. Inoculated cultures were centrifuged at 3000 × g for one hour at 34°C and incubated at 35°C for about one hour. The growth medium and inoculum from each tube was then replaced with 1 ml maintenance medium containing 1 μg cytochalasin and cultures were reincubated for a further two or three days. Cell monolayers were then fixed in methanol, stained with Giemsa, and scanned for typical intracytoplasmic inclusions. If an equivocal result was obtained from a specimen it was cultured again.

When monolayers yielded chlamydial inclusions the number of inclusions per coverslip were assessed by scanning the whole monolayer, when $\leqslant 10^3$ inclusions were present, and by counting inclusions in a proportion of the monolayer, when $>10^3$ inclusions were present.

STATISTICAL ANALYSIS

The significance of the findings in this study was assessed by the χ^2 test.

Results

Specimens were obtained from 919 patients. Processing these specimens occupied one of us (IDP) full-time and one of us (SJR) for 4-8 hours a week, the latter time being spent mainly on the microscopy work. It was usually possible to report the results within 5-8 days of receiving a specimen, the delay (apart from the necessary 2-3 days' incubation) being due largely to the time taken to scan large numbers of preparations by darkground microscopy.

Of the 919 specimens, 10 were inadvertently left at 4°C for 72 hours, 14 were contaminated or were toxic to the cells, and 99 were from patients who had received antimicrobial therapy during the previous month. Valid results were therefore obtained on 796 specimens; only five of these specimens had to be cultured twice before an unequivocal result was obtained.

COMPARISON OF CULTURE TECHNIQUES

"Holding" before inoculation

Chlamydia were isolated from 37 (19%) of 196 unfrozen specimens inoculated into fresh cytochalasin-treated McCoy cell cultures compared with 35 (23%) of 155 unfrozen specimens inoculated into cultures held at room temperature for 24 hours and 14 (15%) of 95 unfrozen specimens inoculated into cultures held at room temperature for 48 hours (Table I). The difference between these isolation rates was not statistically significant ($\chi^2_2 = 3\cdot574$, $0\cdot1 < P < 0\cdot2$) indicating that cultures kept at room temperature for 24 or 48 hours before inoculation are

TABLE I *Results of chlamydial isolation in fresh McCoy cell cultures and in cultures held at room temperature for 24 or 48 hours before inoculation*

Cytochalasin-treated McCoy cell cultures	*Positive specimens out of total tested*†	
	No	*%*
Fresh*	37/196	19
Held at room temperature for		
24 hours	35/155	23
48 hours	14/95	15

*Cell cultures inoculated after three-day treatment with cytochalasin
†Unfrozen specimens

as satisfactory for growth of chlamydia as fresh cultures.

Freezing before inoculation

Storage of specimens at −70°C before inoculation had no effect on the chlamydial isolation rate. Thus chlamydia were isolated from 68/350 (19%) of frozen specimens compared with 86/446 (19%) of unfrozen specimens. Moreover, when chlamydia were isolated, the yields of inclusions from the frozen and unfrozen specimens were similar, indicating there had been no loss of infectivity in the frozen specimens (Table II). Overall, 40 (26%) of 154 positive specimens yielded <10 inclusions/coverslip and 30 (19·5%) specimens yielded $>10^3$ inclusions/coverslip.

ISOLATION IN WOMEN WITH GONORRHOEA AND IN FEMALE PARTNERS OF MEN WITH NGU

N gonorrhoeae was cultured from 71 patients at the time specimens for isolation of chlamydia were obtained, and chlamydia were recovered from 34 (48%) of these patients. One hundred and forty-two patients were partners of men with NGU and chlamydia were isolated from 52 (37%) of these women. Chlamydia were isolated from 68 (12%) of the remaining 573 patients.

MANAGEMENT OF CHLAMYDIAL INFECTIONS

All patients from whom *C trachomatis* was recovered were treated with a course of tetracyclines (normally oxytetracycline 1 g/day for 10 days). All women who were partners of men with NGU received treatment before the chlamydial isolation result was known; most other patients infected with chlamydia received their treatment as a result of the laboratory report.

Discussion

Various modifications of the technique of isolating chlamydia in cytochalasin-treated McCoy cells enabled a large number of specimens (60-80/week) to be tested in this study. By holding cell cultures at room temperature for 24-48 hours before inoculation it was necessary to prepare cell cultures only twice a week; inoculation of clinical specimens into the growth medium of the cell cultures and subsequent replacement of both inoculum and growth medium with maintenance medium after centrifugation produced monolayers of good quality which were easy to scan by darkground microscopy and which rarely yielded equivocal results; the use of one rather than two cell cultures for each specimen halved the number of cultures required, while storage of clinical specimens at −70°C without loss of infectivity meant that a particular isolate could be retrieved and passaged in McCoy cells if this was necessary for serotyping or other purposes.

Previous studies have shown consistently that chlamydia are isolated significantly more often from women with gonorrhoea and from women who are consorts of men with NGU than from other women attending STD clinics.[6 7 11-13] In these earlier studies infections with *T vaginalis* and *C albicans*—organisms which predominantly infect the vagina rather than the endocervix—had no significant effect on the recovery of chlamydia. The chlamydial isolation rates in women infected with *T vaginalis* and *C albicans* have therefore not been analysed in this present study. The isolation rates of 37% in consorts of men with NGU, of 48% in women with gonorrhoea, and of 12% in the remainder of the women studied are comparable to those reported in the earlier studies.

Overall the isolation rate was lower in the present study than in that reported previously from the same clinic.[6] This was probably due partly to the fact that in the earlier study 20% of the women had gonorrhoea compared with only 9% in the present survey. Since women with gonorrhoea are so frequently infected with chlamydia, reduction in the rate of gonococcal isolation in a given group of women would also be associated with a lower chlamydial isolation rate. In addition, the chlamydial isolation rate in women with gonorrhoea was lower in the present study (48%) than that reported earlier (63%), although this difference was not statistically significant ($\chi^2 = 2\cdot39$, $0\cdot1 < P < 0\cdot2$). Irradiated

TABLE II *Comparison of yields of inclusions from positive specimens stored at −70°C and positive specimens kept at 4°C for 24 hours or less*

	No of positive specimens (%)				
	*Inclusions/coverslip**				
	<10	*10-99*	*100-999*	*>1000*	*Total*
Stored at −70°C	18 (26)	19 (28)	15 (22)	16 (24)	68 (100)
Kept at 4°C for <24 hr	22 (26)	29 (34)	21 (24)	14 (16)	86 (100)
Total	40 (26)	48 (31)	36 (23·5)	30 (19·5)	154 (100)

*Each culture inoculated with 0·25 ml of clinical specimen

McCoy cells rather than cytochalasin-treated McCoy cells were used in the earlier study; however, the range of inclusion counts, in particular the proportion of positive specimens yielding $>10^3$ inclusions per coverslip, was very similar in the two studies (20% in 1974 compared with 19·5% in the present work) indicating that the sensitivity of the two isolation procedures was similar.

There is convincing evidence at present that not only are genital chlamydia pathogens in the male urethra and cervix but that by ascent of the genital tract or by transfer to the eyes and respiratory tract they can also cause serious disease elsewhere.[14-20] Identification and eradication of these infections, particularly the large reservoir of infection in the genital tract, is therefore undoubtedly desirable. The present work allowed 158 genital chlamydial infections to be identified and treated during the three-month study period. Without isolation studies these infections would have been unrecognised, and, in women who were not partners of men with NGU, they would have remained untreated. Since this study ended, chlamydial isolation efforts in this clinic have therefore been concentrated on the latter group of patients.

This study confirms the high prevalence of chlamydial infections in women with gonorrhoea as well as in female partners of men with NGU; in a given group of women the overall chlamydial isolation rate will be significantly influenced by the proportion of these women in the total group. This study also raises the question of whether or not male partners of women with cervical chlamydial infections, not known to have non-specific urethritis, should be traced and treated with tetracyclines on epidemiological grounds.

Although present cell culture techniques for isolation of chlamydia are too cumbersome to allow mass screening of the large numbers of patients who presently attend STD clinics in the UK, screening of selected groups of patients is both feasible with limited laboratory facilities and of value in identifying and eradicating genital chlamydial infections which would not otherwise be treated.

We are grateful to the nursing and medical staff of the Bristol Royal Infirmary STD clinic for their co-operation, and to Dr S K R Clarke and Dr A E Jephcott for their helpful comments during the preparation of this manuscript.

References

1. Schachter J. Chlamydial infections (first of three parts). *N Engl J Med* 1978; **298:** 248-435.
2. Schachter J. Chlamydial infections (second of three parts). *N Engl J Med* 1978; **298:** 490-5.
3. Richmond SJ, Oriel JD. Recognition and management of genital chlamydial infection. *Br Med J* 1978; **2:** 480-3.
4. Blackman HJ, Yoneda C, Dawson CR, *et al.* Antibiotic susceptibility of *Chlamydia trachomatis. Antimicrob Agents Chemother* 1977; **12:** 673-7.
5. Ridgway GL, Owen JM, Oriel JD. The antimicrobial susceptibility of *Chlamydia trachomatis* in cell culture. *Br J Vener Dis* 1978; **54:** 103-6.
6. Hilton AL, Richmond SJ, Milne JD, *et al. Chlamydia* A in the female genital tract. *Br J Vener Dis* 1974; **50:** 1-10.
7. Nayyar KC, O'Neill JJ, Hambling MH, *et al.* Isolation of *Chlamydia trachomatis* from women attending a clinic for sexually transmitted diseases. *Br J Vener Dis* 1976; **52:** 396-8.
8. Oriel JD, Johnson AL, Barlow D, *et al.* Infection of the uterine cervix with *Chlamydia trachomatis. J Infect Dis* 1978; **137:** 443-51.
9. Richmond SJ. The isolation of *Chlamydia* subgroup A (*Chlamydia trachomatis*) in irradiated McCoy cells. *Med Lab Technol* 1974; **31:** 7-9.
10. Sompolinsky D, Richmond SJ. Growth of *Chlamydia trachomatis* in McCoy cells treated with cytochalasin B. *Appl Microbiol* 1974; **28:** 912-4.
11. Oriel JD, Powis PA, Reeve P, *et al.* Chlamydial infections of the cervix. *Br J Vener Dis* 1974; **50:** 11-16.
12. Burns DCM, Darougar S, Thin RN, *et al.* Isolation of *Chlamydia* from women attending a clinic for sexually transmitted disease. *Br J Vener Dis* 1975; **51:** 314-8.
13. Ridgway GL, Oriel JD. Interrelationship of *Chlamydia trachomatis* and other pathogens in the female genital tract. *J Clin Path* 1977; **30:** 933-6.
14. Beem MO, Saxon EM. Respiratory tract colonisation and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis. N Engl J Med* 1977; **296:** 306-10.
15. Harnisch JP, Berger RE, Alexander ER, *et al.* Aetiology of acute epididymitis. *Lancet* 1977; **1:** 819-21.
16. Mårdh P-A, Ripa T, Svensson L, *et al. Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977; **296:** 1377-9.
17. Rees E, Tait IA, Hobson D, *et al.* Perinatal chlamydial infections. In: Hobson D, Holmes KK (eds). *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 140-7.
18. Schachter J. The expanding clinical spectrum of infections with *Chlamydia trachomatis. Sex Transm Dis* 1977; **4:** 116-8.
19. Paavonen J, Saikku P, Vesterinen E, *et al. Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis* 1979; **55:** 203-6.
20. Tipple MA, Beem MO, Saxon EM. Clinical characteristics of the afebrile pneumonia associated with *Chlamydia trachomatis* infection in infants less than 6 months of age. *Pediatrics* 1979; **63:** 192-203.

Br J Vener Dis 1980;**56**:96-100.

Colonisation of pregnant and puerperal women and neonates with Chlamydia trachomatis

P-A MÅRDH,* I HELIN,† S BOBECK,‡ J LAURIN,§ AND T NILSSON§

*From the *Institute of Medical Microbiology, University of Lund; the Departments of †Paediatrics and ‡Gynaecology, Landskrona Hospital; and the §Department of Obstetrics and Gynaecology, Helsingborg Hospital, Sweden*

SUMMARY *Chlamydia trachomatis* was cultured from cervical specimens of 14 (16·1%) of 231 women applying for legal abortion and from 23 (8·7%) of 273 puerperal women. The chlamydial isolation rate was related to the women's age. Of the pregnant and puerperal women under 20 years *C trachomatis* was isolated in 10% and 24% respectively; in those aged between 20 and 24 years the rates were 8·7% and 10·2% respectively whereas in those over 24 years the rates were 4·2% in both groups. Chlamydia were isolated more frequently from cervical specimens than from urethral specimens. However, if a cervical specimen alone had been examined the diagnosis would have been missed in three (17%) of 18 women.

IgG antichlamydial antibodies (titre ⩾1/32) were detected by a micro-immunofluorescence test in samples of cord blood from 35 (25%) of 139 infants of the puerperal women. Of the 23 infants born to mothers harbouring chlamydia in the cervix *C trachomatis* was isolated from the conjuntival folds in five (22·5%). The chlamydial isolation rate from the eyes of the neonates was related to the time of sampling. None of the 108 infants examined between 6 and 7 days old was chlamydia-positive whereas chlamydia could be recovered from the conjunctival folds of four of them when re-examined from three to 23 days later.

Introduction

Chlamydia trachomatis is one of the most common pathogens recovered from the genital tract. The organism is generally transmitted by sexual intercourse.[1] It is a frequent cause of serious complications following primary genital infections both in women (namely, acute salpingitis[2]) and in men (namely, acute epididymitis[3]). Asymptomatic female carriers of *C trachomatis* occur at least as frequently as those of *Neisseria gonorrhoeae*.[4 5] However signs of inflammation are generally present in women with genital chlamydial infection.[6]

Chlamydia may be transmitted from mother to infant at delivery. *C trachomatis* has been isolated from the eyes, the nasopharynx, the larynx, the lungs, the vagina, and from faecal specimens of infants born to mothers harbouring the organism in the cervix.[7] Such infection of the infant may result in conjunctivitis,[8 9] which generally runs a mild clinical course. However severe exudative ophthalmitis may occur,[10] and rarely conjunctival scarring and pannus develop as sequelae.[11-13] In recent years, *C trachomatis* has also been established as one of the most common causes of neonatal pneumonia.[14 15] Neonates may also harbour this organism without showing signs of infection.[15]

The present study was performed to: (a) study the incidence of infections with *C trachomatis* and *N gonorrhoeae* in different age groups of pregnant (women applying for legal abortion) and puerperal women; (b) determine the presence of IgG antichlamydial antibodies in cord blood of infants of the mothers studied; and (c) investigate chlamydial colonisation in the eyes of infants born to mothers infected with the organism.

Patients and methods

STUDY POPULATION AND SAMPLING

Group 1

Two hundred and thirty-five women applying for legal abortion (group 1) in the cities of Landskrona and Helsingborg between the eighth and thirteenth week of pregnancy were examined for cervical

Address for reprints: Dr P-A Mårdh, Institute of Medical Microbiology, Sölvegatan 23, S-223 62 Lund, Sweden

Received for publication 3 September 1979

infection with *C trachomatis;* specimens for culture for *N gonorrhoeae* were collected from the urethra, the cervix, and the rectum. All the 235 women studied had abortions performed.

Group 2

Cervical cultures for *C trachomatis* were also obtained from 293 puerperal women (group 2) from the city of Landskrona; samples were taken on the sixth or seventh day after delivery. Specimens for chlamydial isolation were also collected from the urethra in 169 of these women. Specimens for isolation of *N gonorrhoeae* were collected from the urethra, the cervix, and the rectum in 185 women in this group.

Group 3

Specimens were collected from the conjunctival folds of the infants born to the first 108 puerperal women studied (group 3) at six or seven days after birth. Of the remaining 185 puerperal women, conjunctival specimens were collected only from those neonates whose mothers had been found to be infected with chlamydia; samples were collected from these infants between 10 and 30 days after birth.

CULTURE TECHNIQUES

Specimens for chlamydial isolation were collected with a calcium-alginate swab (Inolex Corp, Glenwood, Illinois) by gentle rotation before withdrawal of the swab from the sampling site. The swabs were transported in a sucrose-phosphate buffer (2-SP),[16] containing 5% fetal calf serum, 10 μg gentamycin, 100 μg vancomycin, and 2·5 μg amphotericin B per ml. Specimens were transported chilled (14°C) to the laboratory, where approximately 95% of them were stored at 4°C for less than 48 hours before inoculation on to tissue cultures. The remaining 5% of specimens had been stored at −20°C before being tested. Chlamydial isolation was made on cycloheximide-treated McCoy cells according to a technique described by Ripa and Mårdh,[17] although RPMI 1640 was used as tissue culture medium.

The sampling technique and the methods used for the isolation and identification of *N gonorrhoeae* were those described elsewhere.[18]

SEROLOGICAL TESTS

Specimens of cord blood from 139 of the neonates were tested for IgG antichlamydial antibodies with a modified micro-immunofluorescence (micro-IF) test using pools of antigens as described by Treharne and co-workers.[19]

TREATMENT

Women in group 1 found to be infected with chlamydia were given doxycycline 200 mg (Vibramycin, Pfizer) daily for five days followed by 100 mg daily for 10 days; their sexual consorts received the same treatment. Women in group 2 infected with the organism received doxycycline 100 mg daily for 14 days; the same treatment was prescribed for their husbands. Infants with positive chlamydial isolation from the eyes were given erythromycin 20-25 mg/kg body weight (Abboticin, Abbott) twice daily for 10 days and chloromycetin ointment (Chloromycetin, Parke-Davis) locally.

Results

CULTURAL STUDIES

Group 1

In four (1·7%) of the 236 women in group 1 the cervical culture results were inconclusive owing to a non-specific cytopathogenic effect. *C trachomatis* was isolated from 14 (6·2%) of the remaining 231 women (Table I). Women of less than 20 years had the highest rate of positive culture results (10%) (Table I).

N gonorrhoeae was recovered from two (0·9%) women in group 1, one of whom also harboured chlamydia.

Group 2

Chlamydial cultures from 20 (6·8%) of the 293 women in this group gave an inconclusive result. *C trachomatis* was isolated from 23 (8·4%) of the remaining 273 women. Also in this group the isolation rate was related to age (Table I). Of the women under 20 years 24% harboured the organism compared with 4·4% of those of 25 years or older.

N gonorrhoeae was not isolated from any of the 185 puerperal women.

TABLE I *Isolation of Chlamydia trachomatis from the cervix in different age groups of 231 pregnant (group 1) and 273 puerperal (group 2) women*

Age (yrs)	*Positive chlamydial isolation (out of total in each age range)*			
	*Group 1**		*Group 2**	
	No	*%*	*No*	*%*
<19	2/22	10·0	6/25	24·0
20-24	6/69	8·7	11/108	10·2
25-29	3/49	6·0	3/87	3·4
>30	3/91	3·9	3/53	5·7
Total	14/231	6·1	23/273	8·7

* The four women in group 1 and the 20 women in group 2 with inconclusive culture results have been excluded.

Comparison of cervical and urethral cultures (group 2)

The culture results for the 169 puerperal women from whom specimens for chlamydial isolation were collected from both the cervix and the urethra are shown in Table II. Eighteen patients harboured chlamydia—in most instances in the cervix (15 cases), although specimens from the cervix most often caused a non-specific cytopathogenic effect in the tissue cultures.

TABLE II *Comparison of chlamydial culture results in cervical and urethral specimens from 169 puerperal women (group 2)*

*Culture result**		*No of positive results from both sites*
Cervix	*Urethra*	
–	–	134
+	–	11
–	+	3
+	+	3
Total		151

+ Positive – negative
* The culture result was inconclusive in cervical specimens from 10 patients, in urethral specimens from three patients, and in both specimens from four patients. In one patient the urethral culture was spoiled by bacterial overgrowth but the cervical specimen yielded chlamydia.

Group 3

C trachomatis was not isolated in samples from any of the infants of the first 108 puerperal mothers studied when examined at six or seven days after birth. Nine of their mothers harboured chlamydia in the cervix. The infants of these nine mothers were re-examined between 10 and 30 days after birth as were a further 14 infants of the remaining 185 mothers who were infected with *C trachomatis*. At this time five infants harboured chlamydia in their conjunctival folds, four of whom were not found to be infected when examined at six or seven days after birth (Table III).

SEROLOGICAL STUDIES

The reciprocal titres of IgG antichlamydial antibodies in the 139 cord blood samples studied are shown in Table IV. Antibodies at a titre of 1/32 or greater were found in 25% of the specimens tested.

TABLE III *Chlamydial isolation results from conjunctival folds of neonates at different ages*

Age (days)	*No of infants*		*No of mothers with positive cervical results for chlamydia*
	Examined	*Infected*	
6-7	108	0	9
10-30	23	5	23

TABLE IV *IgG antichlamydial antibodies in cord blood from 139 neonates tested by a micro-immunofluorescence test*

	Antichlamydial antibody (reciprocal of titre)							
	≤16	*32*	*64*	*128*	*256*	*512*	*1024*	*2048*
No of neonates	104	15	11	3	2	2	1	1

Discussion

In this study *C trachomatis* was recovered from 6·1% of the pregnant (women applying for legal abortion during the first trimester) and from 8·4% of the puerperal women studied. All these women were inhabitants of the cities of Helsingborg and Landskrona or of the surrounding rural areas (a total of approximately 200 000 inhabitants). These cities are located by the strait of Öresund in a mixed industrial and agricultural area with close contacts with the metropolitan region of Copenhagen. Both groups of women studied comprised consecutive cases treated at the gynaecological departments in these two cities. In Landskrona, which has 51 000 inhabitants, about 400 children are born annually, and during recent years about 600 women have applied annually for legal abortion in the Helsingborg-Landskrona region.

The chlamydial isolation rate in the women in the present study is similar to that reported in pregnant women in the United States (5-13%).[1 13] These figures are somewhat higher than those for healthy, non-pregnant women who have served as controls in studies of women attending venereal disease clinics; the incidence has usually been less than 5%.[1 20] Moreover, we have isolated the organism in 10-30% of women attending venereal disease and gynaecological clinics in the same county (Skåne) as the women in the present study (Mårdh *et al*, unpublished data).

As shown by the present study, the age distribution of the individuals studied influences the isolation rate of *C trachomatis*. In our series of sexually active persons the younger the women the more frequently the organism was isolated. This was also true in an earlier study of patients attending a gynaecological clinic in the same hospital catchment region[21] and in a recent study of pregnant women in the United States.[13] The comparatively low prevalence of genital chlamydial infection in the older age groups suggests that such infections are often self-limiting or that these women are less sexually active.

In non-pregnant, non-puerperal women with clinical signs of infection of the lower genital tract *C trachomatis* may be found significantly more often than in those without such signs.[6] However, whether or not a pregnant or puerperal women harbours chlamydia is extremely difficult to establish merely by clinical examination alone. The high incidence of asymptomatic female carriers of *C trachomatis*[4 5] indicates that the cervix is an important reservoir for the spread of the organism in the community, as is shown in the present study. None of the chlamydia-positive puerperal women had complained of symptoms suggesting genital infection; this was also true of most of the women who had applied for abortion and who harboured *C trachomatis*.

It has been claimed that *C trachomatis* is a more common cause of sexually transmitted disease than is *N gonorrhoeae*. This claim is supported by the findings in the present study.

A comparison of the results of the cervical and urethral cultures in the puerperal women showed that *C trachomatis* could be recovered most often from cervical specimens. The cervix must therefore be recommended as the sampling site if lack of laboratory facilities restricts the number of specimens that can be investigated. However, a urethral specimen should also be taken if possible from puerperal women, as *C trachomatis* was isolated from the urethra alone in three (17%) of 18 culture-positive women.

The micro-IF test detects species-specific antibodies to *C trachomatis*.[22] Since IgG antibodies pass through the placental barrier it should be possible to study their production in the mother by examining samples of cord blood. The result of our serological study indicated that at least one-third—or 5-6 times as many women as shown by the culture studies—had been exposed to *C trachomatis*. In principle, similar findings have been reported by others.[23]

The risk of a newborn baby acquiring an eye infection due to *C trachomatis*, when the mother is harbouring the organism in the cervix, has been estimated to be between 23 and 60%.[8 13 24-26] In our study, only 22% of the babies of chlamydia-positive mothers harboured chlamydia in their conjunctival folds. Frommel *et al*,[13] in a study of neonates, reported that nasopharyngeal specimens gave a positive result for chlamydia more frequently than specimens from the eyes. In the present study conjuctival specimens were collected from five of the 23 infants of chlamydia-positive mothers, but all gave a negative result.

The results of our study indicate that the time of sampling influences the isolation rate of chlamydia from the eyes of neonates. Four children in our study were chlamydia-negative at six to seven days after birth but harboured chlamydia in their conjunctival folds when re-examined three to 23 days later. From other studies,[9 27] the incubation period of ophthalmia neonatorum caused by chlamydia can be as short as a few days. All the infants in our study had been treated prophylactically with silver nitrate on the day of delivery. Although it is known that Credé prophylaxis does not inhibit the growth of *C trachomatis*,[25] it might have prolonged the incubation period and possibly also modified the clinical course.

None of the neonates harbouring chlamydia in their eyes showed such signs of infection as discharge, reddening, bulging, or oedema. This is in contrast to Rees and his co-workers,[9] who found clinical signs of infection in all of 33 infants infected with chlamydia in their eyes. Because the infection was detected early and treatment was begun as soon as the result of the cultures was known, signs of conjunctivitis may have been prevented from developing.

An increasing number of reports from North America[14 15 25 28] and from Europe[29] have shown that *C trachomatis* may cause pneumonia in neonates. This type of neonatal pneumonia, which may occur with or without signs of conjunctivitis, was not diagnosed in any of our children.

Martin and his co-workers[30] recently reported that the relative risk of stillbirth or neonatal death after 20 weeks' gestation was significantly increased (approximately ten-fold) among chlamydia-positive women compared with chlamydia-negative controls who were matched for age, race, socioeconomic status, and number of pregnancies. Frommel *et al*,[13] however, did not find any correlation between genital chlamydial infection in pregnant women on one hand and difficulties of labour and delivery, initial condition of the neonate, and postpartum complications on the other.

The incidence of post-abortion and puerperal complications of primary genital chlamydial infections could not be established in the present study, as all carriers of *C trachomatis*, as well as their sexual consorts, were treated with doxycycline. In venereal disease clinic patients, this treatment is known to eradicate *C trachomatis* effectively. However, in another study (Møller *et al*, unpublished observations) of women who underwent legal abortion and who received no antibiotic treatment, we found retrospectively that acute salpingitis had developed in several patients whose cervical specimens collected before abortion had yielded chlamydia. In some of these patients, we have evidence that the salpingitis was caused by *C trachomatis*. Thus, the diagnosis of genital chlamydial infection in women in whom abortion is performed and treatment with antibiotics if the women are chlamydia-positive are clearly important.

The high incidence of genital chlamydial infection in pregnant women suggests that steps should be taken routinely to detect such infections, particularly in young women. At present, however, limited laboratory resources make this difficult. Further studies are also needed before any recommendations can be made as to whether or not antibiotic treatment should be given to all pregnant women who are found to be carriers of *C trachomatis*.

The infants harbouring chlamydia in their eyes were treated with oral erythromycin and chloromycetin ointment to prevent bacterial secondary infections as soon as the culture results were known. No control cultures were performed, but none of the infants developed signs of conjunctivitis or pneumonia during the follow-up period of three to 18 months. Although chlamydial conjuctivitis and pneumonia seem generally to be self-limiting the risk that the infant might develop severe eye infection, or even sequelae, seems to justify general treatment with erythromycin in infants in whom *C trachomatis* has been recovered. Viral pneumonia during infancy holds a high risk of respiratory-tract complications during later life;[31 32] whether or not this is also true of pneumonia caused by *C trachomatis* is not known. Antibiotic treatment may also prevent the transmission of chlamydia to other members of the family.[33]

This study was supported by grant 16X-4509 from the Swedish Medical Research Council.

References

1. Schachter J. Chlamydial infections. *N Engl J Med* 1978; **298:** 428-35; 490-5; 540-9.
2. Mårdh P-A, Ripa T, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977; **296:** 1377-9.
3. Berger RE, Alexander ER, Harnisch JP, Paulsen CA, Mondo GD, Ansell J, Holmes KK. Etiology, manifestations and therapy of acute epididymitis: Prospective studies of 50 cases. *J Urol* 1979; **121:** 750-4.
4. MacD Burns DC, Darougar S, Thin RN, Lothian L, Nicol CS. Isolation of *Chlamydia* from women attending a clinic for sexually transmitted diseases. *Br J Vener Dis* 1975; **51:** 314-8.
5. Nayyar KC, O'Neill JJ, Hambling MH, Waugh MA. Isolation of *Chlamydia trachomatis* from women attending a clinic for sexually transmitted diseases. *Br J Vener Dis* 1976; **52:** 396-8.
6. Paavonen J, Saikku P, Vesterinen E, Meyer B, Vartiainen E, Saksela E. Genital chlamydial infections in patients attending a gynecological outpatient clinic. *Br J Vener Dis* 1978; **54:** 257-61.
7. Schachter J, Dawson CR. Is trachoma an ocular component of a more generalized chlamydial infection? *Lancet* 1979; **1:** 702-3.
8. Chandler JW, Alexander ER, Pheifer TA, Wang S-P, Holmes KK, English M. Ophthalmia neonatorum associated with maternal chlamydial infections. *Trans Am Acad Ophthalmol Otolaryngol* 1977; **83:** 302-8.
9. Rees E, Tait IA, Hobson D, Byng RE, Johnson FWA. Noenatal conjunctivitis caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. *Br J Vener Dis* 1977; **55:** 173-9.
10. Jones BR. Prevention of blindness from trachoma. *Trans Ophthalmol Soc UK* 1975; **95:** 16-33.
11. Watson PG, Gairdner D. TRIC agent as a cause of neonatal eye sepsis. *Br Med J* 1968; **3:** 527-8.
12. Nordhorst CH, Dawson C. Sequalae of neonatal inclusion conjunctivitis and associated disease in parents. *Am J Ophthalmol* 1971; **71:** 861-7.
13. Frommel GT, Rothenberg R, Wang S-P, *et al.* Chlamydial infection of mothers and their infants. *J Pediatr* 1979; **95:** 28-32.
14. Beem MO, Saxon EM. Respiratory tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. *N Engl J Med* 1977; **293:** 306-10.
15. Tipple MA, Beem MO, Saxon EM. (1979). Clinical characteristics of the afebrile pneumonia associated with *Chlamydia trachomatis* infection in infants less thán 6 months of age. *J Pediatr* 1979; **63:** 192-7.
16. Gordon FB, Harper IA, Quan AL, Treharne JD, Dwyer RSt C, Garland JA. Detection of *Chlamydia (Bedsonia)* in certain infections of man. I Laboratory procedures, comparison of yolk sac and cell culture detection and isolation. *J Infect Dis* 1969; **120:** 451-62.
17. Ripa KT, Mårdh P-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol* 1977; **6:** 328-31.
18. Mårdh P-A, Mårtensson D, Soltesz LV. A new effective culture medium for *Neisseria gonorrhoeae*. *Sex Transm Dis* 1978; **5:** 10-3.
19. Treharne JD, Darougar S, Simmons PD, Thin RN. Rapid diagnosis of chlamydial infection of the cervix. *Br J Vener Dis* 1978; **54:** 403-8.
20. Hilton AL, Richmond SJ, Milne JD, Hindley F, Clarke SKR. *Chlamydia A* in the female genital tract. *Br J Vener Dis* 1974; **50:** 1-10.
21. Ripa KT, Svensson L, Mårdh P-A, Weström L. *Chlamydia trachomatis* cervicitis in gynecologic outpatients. *Obstet Gynecol* 1978; **52:** 698-702.
22. Wang S-P, Graystone JT. Human serology in *Chlamydia trachomatis* infection with microimmunofluorescence. *J Infect Dis* 1974; **130:** 388-97.
23. Treharne JD, Darougar S, Jones BR. Modification of the microimmunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977; **30:** 510-7.
24. Alexander ER, Chandler J, Pheifer TA, Wang S-P, English M, Holmes KK. Prospective study of perinatal *Chlamydia trachomatis* infection. In: Hobson D, Holmes K, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 148-52.
25. Schachter J, Dawson CR. Inclusion conjunctivitis of the newborn and chlamydial pneumonia in infants. In: Schachter JS, Dawson CR, eds. *Human Chlamydial Infections.* Littleton: PSG Publishing Company, 1978: 111-20.
26. Lumicao GG, Gyves MT, Stuart LA, Heggie AD. Prospective study of perinatal infections with *Chlamydia trachomatis*. *Pediatr Res* 1979; **13:** 464.
27. Freedman A, Al-Hussaini MK, Dunlop EMC, *et al.* Infection by TRIC agent and other members of the Bedsonia group: with a note on Rieter's disease. *Trans Ophthalmol Soc UK* 1966; **86:** 313-20.
28. Beem MO, Saxon E, Tipple MA. Treatment of chlamydial pneumonia of infancy. *J Pediatr* 1979; **63:** 198-203.
29. Hallberg A, Mårdh P-A, Persson K, Ripa T. Pneumonia associated with *Chlamydia trachomatis* infection in an infant. *Acta Paediatr Scand* 1979; **68:** 765-7.
30. Martin DH, Alexander ER, Eschenbach *et al.* Prospective study of chlamydia infection in pregnancy. *Abstract C3.* In: Proceedings of Sexually Transmitted Diseases, 2nd meeting, Helsinki, Finland, 9-10 August 1979.
31. Rooney JC, Williams HE. The relationship between proven viral bronchiolitis and subsequent wheezing. *J Pediatr* 1971; **79:** 744-7.
32. Burrows B, Knudson RJ, Lebowitz MD. The relationship of childhood respiratory illness to adult obstructive airway disease. *Am Rev Respir Dis* 1977; **115:** 751-60.
33. Jones BR. Ocular syndromes of TRIC virus infection and their possible genital significance. *Br J Vener Dis* 1964; **40:** 3-18.

Cryosurgery: a natural choice.

Recent developments by Spembly have resulted in a range of equipment bringing the benefits of cryosurgery to the treatment of genital warts.

With remarkable results.

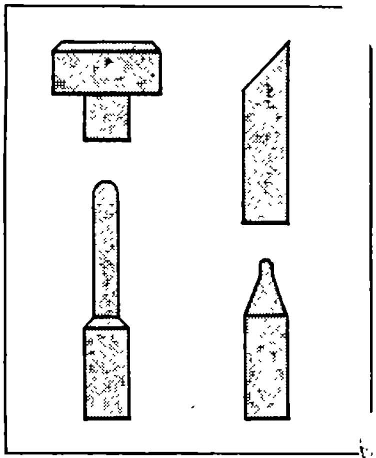
It's a relatively painless, bloodless, approach with a high degree of patient acceptability.

That's why cryosurgery is becoming a natural choice for an ever growing number of physicians.

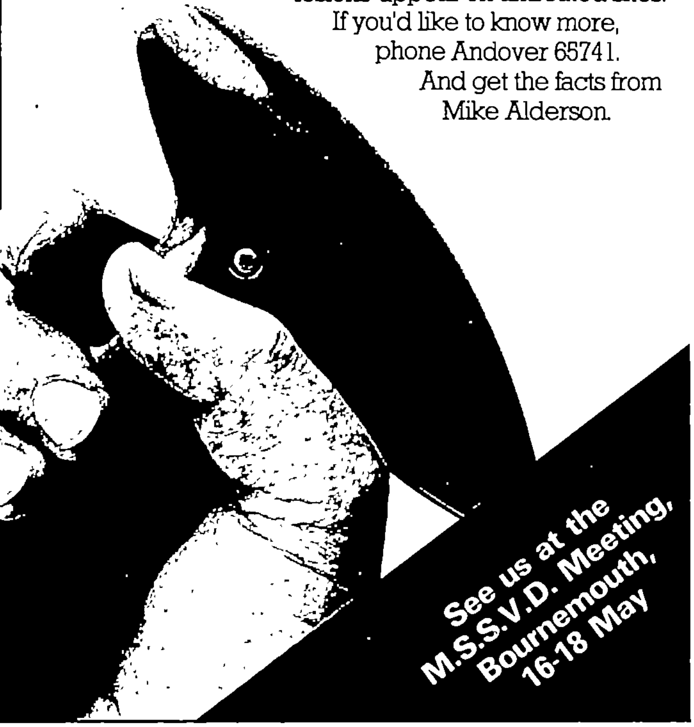
Spembly's range is well suited to the ablation of genital warts, where extensive areas can be treated with no local anaesthetic. The technique is easily repeated should new lesions appear on untreated sites.

If you'd like to know more, phone Andover 65741.

And get the facts from Mike Alderson.



Some of the wide range of interchangeable tips for use with the illustrated Cryogun.



Spembly

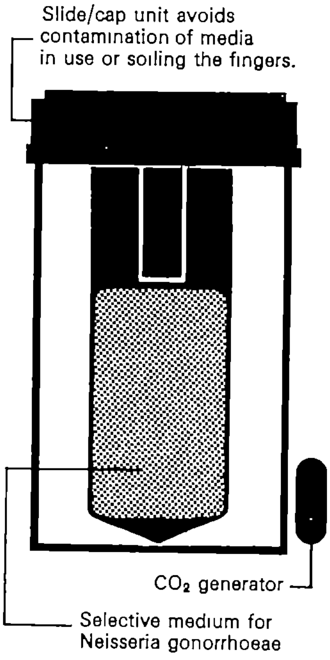
Spembly Limited
Newbury Road Andover Hampshire SP10 4DR England
telephone (0264) 65741 telex 47403

NEW

Till-U-Test G.C. Slide

for simple culture of ***Neisseria gonorrhoeae***

- **Incubation problems solved by self-contained unit with built-in anaerobic/CO_2 environment**
- **No transport medium needed**
- **Results in 24-48 hours**
- **Selective culture medium for *N. gonorrhoeae***
- **Economic in use.**


Slide/cap unit avoids contamination of media in use or soiling the fingers.
CO₂ generator
Selective medium for Neisseria gonorrhoeae

Further information from:

Tillomed Laboratories Ltd.
Henlow Trading Estate, Henlow, Beds.

Br J Vener Dis 1980;**56**:101-4.

Treatment of nongonococcal urethritis with trimethoprim-sulphadiazine and with placebo

A double-blind partner-controlled study

J PAAVONEN,* M KOUSA,† P SAIKKU,‡ E VARTIAINEN,* L KANERVA,† AND A LASSUS†

*From the *I-II Departments of Obstetrics and Gynaecology and the †Department of Dermatology and Venereology, Helsinki University Central Hospital; and the ‡Department of Virology, University of Helsinki, Finland*

SUMMARY The effect of sulphadiazine and trimethoprim (T-S) was compared with that of a placebo in the treatment of 75 men with nongonococcal urethritis (NGU) and their female sexual partners. Forty (53%) men and 30 (40%) women had positive culture results for *Chlamydia trachomatis* before treatment was started. Thirty-four patients of each sex received active treatment. Of these, 19 men and 15 women had positive culture results for *C trachomatis* before treatment with T-S and one of each sex had a positive culture result after treatment. *C trachomatis* had been isolated in 21 men and 15 women before treatment with placebo. At follow up four weeks after treatment had been started, 16 men and 15 women still harboured *C trachomatis*. Five of the chlamydia-positive patients treated with placebo developed complications (epididymitis, arthritis, or salpingitis) so the trial was discontinued.

Introduction

In a critical review of the treatment of non-specific urethritis Simpoulos[1] suggested that only by comparing the results of treatment with tetracyclines and placebos or other drugs—for instance those outside the antibiotic range and with no suspected side effects—would it be reasonable to form a firm view on the desirability or effectiveness of the treatment. Furthermore, such a comparison should be carried out over a prolonged period of time.[1]

Tetracyclines and erythromycin are the drugs of choice for the treatment of nongonococcal urethritis (NGU), both in chlamydia-positive and chlamydia-negative cases.[2] The frequently simultaneous presence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genital infections of both men and women calls for new antimicrobial agents which are effective against both these micro-organisms. Although both tetracyclines and erythromycin are effective against *C trachomatis, N gonorrhoeae* is frequently less sensitive. Penicillins and spectinomycin, commonly used for the treatment of gonorrhoea, are ineffective against *C trachomatis.*

In recent years several reports have emphasised the effectiveness of trimethoprim-sulphamethoxazole in the treatment of gonorrhoea.[3-9] Carroll and Nicol[10] and Willcox and Sparrow[11] reported favourable results with trimethoprim-sulphamethoxazole in the treatment of NGU. In-vitro studies of cell cultures have shown that genital strains of *C trachomatis* are susceptible to sulphamethoxazole.[12 13] Trimethoprim alone has proved to be ineffective against *C trachomatis* in vitro[13]

The present study was carried out to compare the effect of a new combination of sulphadiazine and trimethoprim with that of a placebo in the treatment of male patients with NGU and their female sexual partners.

Patients and methods

STUDY POPULATION

Seventy-five men with NGU and 75 women, who were their regular sexual partners, were studied during the period from January to November 1978. All male patients were examined and treated at the

Address for reprints: Dr M Kousa, Department of Dermatology and Venereology, Helsinki University Central Hospital, Snellmaninkatu 14, SF-00170 Helsinki 17, Finland

Received for publication 18 June 1979

Outpatient Department for Venereal Diseases and their female partners at the Outpatient Clinics of the First and Second Departments of Obstetrics and Gynaecology, Helsinki University Central Hospital.

DIAGNOSIS

The diagnosis of NGU was established by the presence of urethral discharge, urethral discomfort, >10 leucocytes per high-power field in Gram-stained smears, and negative culture results for *N gonorrhoeae.*[2] Culture for *N gonorrhoeae* was performed for all male and female patients as described earlier.[5] Specimens for the isolation of *C trachomatis* were collected with a sterile cotton-wool swab from both the urethra and the endocervix and placed in transport medium (2-SP). Isolation attempts were made in irradiated McCoy cells as described elsewhere.[14]

TREATMENT

Both partners received the same treatment, either trimethoprim-sulphadiazine (Ditrim duplo®, Neofarma, Helsinki, Finland) or placebo, one tablet twice daily for two weeks. Tablets containing trimethoprim (160 mg) and sulphadiazine (500 mg) or placebo were packed identically and randomised. The code was kept by an independent observer until the trial was completed. The patients were instructed not to have sexual intercourse without the use of a condom until re-examination at two weeks after the end of the treatment. The female partners were examined one to three days after the men, but the treatment of both partners was always started simultaneously.

All the female patients and those male patients with a positive chlamydial culture result initially or with urethral discharge or urethral discomfort or both at follow up were re-examined for the presence of *C trachomatis*. Before treatment was given all patients were informed that it might not be effective and that further treatment might be necessary. Patients who continued to be chlamydia-positive or to show evidence of urethritis when re-examined were retreated with doxycycline (Doximycin®, Orion) 100 mg twice daily for 10 days.

STATISTICAL ANALYSIS

Statistical comparisons were made using the χ^2 method with Yates's corrections.

Results

ISOLATION

C trachomatis was isolated in 40 (53%) men and in 30 (40%) women. All male patients and their partners had negative culture results for *N gonorrhoeae*. In 48 cases the present episode of NGU was the first. In five cases NGU manifested itself as a postgonococcal condition; five patients also had Reiter's disease and one acute prostatitis simultaneously.

TREATMENT

Men

A total of 34 men received trimethoprim-sulphadiazine (T-S) and 41 received a placebo (Table I). Before treatment, *C trachomatis* had been isolated in 19 (56%) of the former group and in 21 (51%) of the latter. At re-examination four weeks after the beginning of treatment one of the patients treated with T-S had not responded to treatment. In the placebo group, 21 treatment failures occurred and 16 patients continued to be chlamydia-positive when re-examined. In five cases of clinical failure after placebo treatment no attempt was made to reisolate *C trachomatis* at follow up. The difference in effect between T-S and placebo on *C trachomatis* was highly significant ($\chi^2_1 = 19\cdot75$, $P<0\cdot001$).

TABLE I *Treatment of male patients with NGU and their female sexual partners with trimethoprim-sulphadiazine and with placebo*

	Chlamydial culture results				
	Before treatment		*After treatment*		*No cultures*
Treatment	+	−	+	−	*performed*
Male patients					
T-S	19 (56%)	15*	1	13	20
Placebo	21 (51%)	20*	16†	2	23
Female partners					
T-S	15 (44%)	19	1‡	32	1
Placebo	15 (37%)	26	15§	32	4

+ Positive − negative

*Men with chlamydia-negative NGU were not systematically assessed by culture after treatment

†Two patients were chlamydia-negative before treatment

‡Initially chlamydia-negative (partner chlamydia-positive)

§Three patients were chlamydia-negative before treatment

T-S = trimethoprim-sulphadiazine

Women

Thirty-four women received T-S and 15 (44%) were chlamydia-positive before treatment (Table I). Thirty-three of them attended for re-examination and 32 had a post-treatment negative culture result for *C trachomatis*. One women who was chlamydia-negative initially became chlamydia-positive during the follow-up period. Her male partner was chlamydia-positive both before and after treatment. Forty-one women were given placebo and 15 (37%) of these had positive culture results for *C trachomatis* before treatment. *C trachomatis* continued to be isolated from 12 of these 15 women, as well as from three others, at follow up. T-S had a significantly better effect on *C trachomatis* in women than did the placebo ($\chi^2_1 = 11\cdot87$, $P<0\cdot001$).

COMPLICATIONS

Complications were observed in the group treated with placebo at follow up. One of the men had developed acute arthritis and one acute epididymitis. Both of these patients harboured *C trachomatis* before and after treatment. Acute salpingitis was diagnosed in three (20%) of the 15 chlamydia-positive women treated with placebo. Because of the complications the trial was discontinued at this stage.

RETREATMENT

Twenty-three men (three from the group treated with T-S and 20 from the group treated with placebo) who either remained chlamydia-positive or showed clinical failure despite negative culture results were treated with doxycycline. Eleven of these men attended for further follow up, and only one was chlamydia-positive and clinically not cured (Table II). The female partner of the only patient with NGU with treatment failure was repeatedly chlamydia-negative. Fifteen of the female partners of the male patients treated with doxycycline attended for further follow up and were all chlamydia-negative.

TABLE II *Results of doxycycline treatment in patients who failed to respond to trimethoprim-sulphadiazine and to placebo*

	Chlamydial culture results				
	Before treatment		*After treatment*		
	+	− *(or no culture performed)*	+	−	*No culture performed*
Men with NGU	17	6	1	10	12
Female partners	15	0	0	15	0

+ Positive − negative

Discussion

It is generally agreed that approximately half of the cases of NGU are caused by *C trachomatis*. Controversy still surrounds the aetiology of chlamydia-negative NGU. There is no doubt that tetracyclines and erythromycin are the most effective antimicrobials for practical use in chlamydial infections, but the effect of these antibiotics in chlamydia-negative NGU is not generally understood. However, many studies support the concept that both tetracyclines and erythromycin are equally effective in chlamydia-positive and chlamydia-negative NGU.[2 13 15]

The criteria for cured NGU are the eradication of symptoms and signs of urethritis together with a negative culture result for *C trachomatis*. There is still no general agreement on the length of the follow-up period after treatment of NGU. Very long follow-up periods have been advocated by some,[16] but examination months after treatment is unreliable because of high default rates, spontaneous remissions, and reinfections. Furthermore, it is not common practice to re-examine patients with uncomplicated gonorrhoea many months after treatment.

Studies of placebo treatment of infections caused by known pathogens, and with obvious risks of complications, are of doubtful value. Such studies of the treatment of NGU have been carried out previously and have shown placebo treatment to be less effective than active treatment.[17 18]

The present study was undertaken to evaluate the difference between placebo and active therapy in a well controlled series with treatment of both partners. The study was indicated because of doubts about the effect of present treatment of NGU[1] and because the need to treat NGU at all seems to be in question.

The chlamydial isolation rate in men with NGU and in their female partners was high in the present series. The results showed that the combination of trimethoprim and sulphadiazine given for two weeks was effective in treating chlamydial NGU. Compared with the results in the placebo group the difference was statistically highly significant. The treatment of the sexual partner was even more convincing. None of the initially chlamydia-positive women remained culture-positive after active treatment.

The results in the placebo group confirmed the good reproducibility of the isolation technique for *C trachomatis*, since over 80% of the initially chlamydia-positive patients still had positive chlamydial culture results four weeks after the placebo treatment had been started. Conversely, only five initially chlamydia-negative patients were found to be chlamydia-positive after placebo treatment.

The high rate of complications in patients treated with placebo in the present series caused the trial to be discontinued. All the complications occurred in chlamydia-positive patients. The risk of ascending infection in women with cervical chlamydia was unexpected; three out of 15 patients treated with placebo developed acute salpingitis.

Thus the trimethoprim-sulphadiazine combination used in the present study is effective in the treatment of chlamydial urogenital infections provided the sexual partner is treated simultaneously. The cure rate for NGU was higher than with trimethoprim-sulphamethoxazole, as reported by Danielsson and Wikström.[4] However, in their study *C trachomatis* was not isolated and sexual partners were not treated. The high incidence of complications in patients treated with placebo in the present series serves as a clear warning against future placebo trials in chlamydial infections.

This study was financially supported by the Finnish Medical Foundation, Helsinki, and Orion Pharmaceuticals Co, Helsinki, Finland.

References

1. Simpoulos JCh. Tetracycline treatment for non-specific urethritis. *Br J Vener Dis* 1977; **53**: 230-2.
2. Lassus A, Paavonen J, Kousa M, Saikku P. Erythromycin and lymecycline treatment of different duration in *C trachomatis* culture-positive and culture-negative nongonococcal urethritis. *Acta Derm Venereol (Stockh)* 1979; **59**: 278-81.
3. Arya OP, Pearson CH, Rao SK, Blowers R. Treatment of gonorrhoea with trimethoprim-sulphamethoxazole in Uganda. *Br J Vener Dis* 1970; **46**: 214-6.
4. Danielsson D, Wikström KJ. Diagnostic aspects of gonococcal and nongonococcal urethritis. Results of treatment with trimethoprim-sulphamethoxazole. In: Danielsson D, Juhlin L, Mårdh P-A (eds). *Genital Infections and Their Complications*. Stockholm: Almqvist & Wiksell International, 1975: 299.
5. Lassus A, Renkonen O-V. Short-term treatment of gonorrhoea with intramuscular and oral forms of trimethoprim-sulphamethoxazole. *Br J Vener Dis* 1979; **55**: 24-5.
6. Svindblad HB. Treatment of gonorrhoea with sulphamethoxazole-trimethoprim. *Br J Vener Dis* 1973; **49**: 50-3.
7. Ullman S, Noirdson A-M, Zachariae H. Trimethoprim-sulphamethoxazole in gonorrhoea. *Acta Derm Venereol (Stockh)* 1971; **51**: 394-6.
8. World Health Organisation. *Neisseria gonorrhoeae* and gonococcal infections. Report of a WHO Scientific Group, Series 616. Geneva: World Health Organisation, 1978: 97.
9. Wright JM, Grimble AS. Sulphamethoxazole combined with 2-4-diaminopyrimidines in the treatment of gonorrhoea. *Br J Vener Dis* 1970; **46**: 34-6.
10. Carroll BRT, Nicol CS. Trimethoprim-sulphamethoxazole in NGU and gonorrhoea. *Br J Vener Dis* 1970; **46**: 31-3.
11. Willcox RR, Sparrow RW. Cotrimoxazole in the treatment of nongonococcal urethritis. *Acta Derm Venereol (Stockh)* 1974; **54**: 317-20.
12. Kuo C-C, Wang S-P, Grayston JT. Antimicrobial activity of several antibiotics and a sulfonamide against *Chlamydia trachomatis* organisms in cell culture. *Antimicrob Agents Chemother* 1977; **12**: 80-3.
13. Treharne JD, Yeo CK, Jones BR, Squires S. Susceptibility of chlamydiae to chemotherapeutic agents. In: Hobson D, Holmes KK (eds). *Nongonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977: 214.
14. Kousa M. Saikku P, Richmond S, Lassus A. Frequent association of chlamydial infection with Reiter's syndrome. *Sex Transm Dis* 1978; **5**: 57-61.
15. Terho P. Treatment of chlamydia-positive and chlamydia-negative nonspecific and postgonococcal urethritis. *Ann Clin Res* 1978; **10**: 299-302.
16. Evans BA. The role of tetracyclines in the treatment of non-specific urethritis. *Br J Vener Dis* 1977; **53**: 40-3.
17. Grimble AS, Amarasuriya KL. Non-specific urethritis and tetracyclines. *Br J Vener Dis* 1975; **51**: 198-205.
18. Prentice MJ, Taylor-Robinson D, Csonka GW. Non-specific urethritis. A placebo-controlled trial of minocycline in conjunction with laboratory investigations. *Br J Vener Dis* 1976; **52**: 269-75.

Br J Vener Dis 1980;**56**:105-6.

Carcinoma of the bladder presenting as gonococcal cystitis

A L BLACKWELL AND J BARROW
From the Department of Genitourinary Medicine, St Thomas's Hospital, London

SUMMARY A patient with gonococcal infection presented with frank haematuria. On subsequent investigation a transitional cell carcinoma was found. Because the haematuria resolved rapidly after treatment with ampicillin no sinister cause for the bleeding was suspected. Since gonorrhoea is rarely associated with gross haematuria, when it does occur a thorough investigation should be undertaken.

Introduction

Haematuria is an unusual presenting feature in cases of gonococcal infection. In a survey of patients attending the genitourinary departments at the London Hospital and St Mary's Hospital haematuria was the presenting symptom in 1% of 200 male patients with gonococcal urethritis.[1] Amarasuriya[2] found only seven cases of gonorrhoea associated with haematuria during a 10-month study of all patients attending Guy's Hospital department of genitourinary medicine. Barlow and Phillips[3] found only one patient with haematuria out of 570 cases of gonococcal infection in women attending St Thomas's Hospital in 1976.

In this paper we report the case of a male patient who complained of haematuria and was found to have both gonococcal infection and a bladder tumour.

Case report

PREVIOUS HISTORY

A 28-year-old Jamaican man attended this department on 10 November 1978. He complained of a three-day history of yellow urethral discharge associated with mild dysuria. He had had frequency of micturition during the 12 hours before attending the clinic but was not worried until he started passing blood instead of urine three hours before attending. He had had three previous episodes of haematuria, in 1969, 1972, and 1973. All these episodes resolved rapidly and spontaneously, but on the last occasion he attended the casualty department of Dudley Road Hospital in Birmingham, where a mid-stream urine (MSU) sample was obtained and the patient referred to the urology outpatient clinic.

The MSU specimens collected on 13 and 20 February 1973 both showed numerous red blood cells but no increase in the number of leucocytes. Both urine cultures gave negative results, and blood urea and electrolyte concentrations were within normal limits. Haemoglobin concentration was 15·4 g/dl and the ESR was 6 mm in the first hour. The patient did not attend for follow up and an intravenous urogram was therefore not carried out.

The patient did not notice any further bleeding until he attended this hospital in November 1978. The history contained no other notable features and no account of trauma to either genitals or loins. He had last had intercourse four days previously with a casual partner and three weeks previously with a regular girlfriend.

CLINICAL EXAMINATION

On examination he was fit but anxious. His blood pressure was 125/80 mm/Hg. A slight, grey urethral discharge was present when the anterior urethra was massaged, but there was no blood or sign of trauma at the meatus. Testes and epididymes were normal and there was no renal angle tenderness.

LABORATORY FINDINGS

A urethral smear showed typical Gram-negative intracellular diplococci and culture for *Neisseria gonorrhoeae* gave a positive result. Urine was uniformly red in three glasses and indistinguishable from blood to the naked eye. An MSU sample showed many red blood cells, but on culture there was a scanty growth of probable contaminants. Haemoglobin was 15·6 g/dl and white blood count

Address for reprints: Dr A L Blackwell, Department of Genitourinary Medicine, St Thomas's Hospital, London SE1 7EH

Received for publication 21 August 1979

was 6·2 × 10^9/l with a normal differential blood count. ESR was 5 mm in the first hour. Sickle-cell test gave a negative result and blood urea and electrolyte concentrations were within normal limits.

TREATMENT

Ampicillin 2 g plus probenecid 1 g were given at once by mouth followed by ampicillin 500 mg plus probenecid 500 mg four times daily for one week.

OUTCOME

The patient was asymptomatic by the following day, and when next seen on 13 November his urine was clear and showed no blood on dip-testing. Urethral cultures now gave negative results. An intravenous urogram was performed on 18 December, which showed a filling defect at the base of the bladder. Unfortunately the patient changed his address and failed to return for urgent cystoscopy. As a result of persistent contact-tracing the patient attended again in May 1979.

On 11 May 1979 he returned to the clinic and although asymptomatic was found to have non-specific urethritis, which was treated with triple tetracycline 1 tablet twice daily for 20 days. He had had no further bleeding since his attack of gonorrhoea in November and his urine still showed no blood on dip-testing.

On 23 May 1979 cystourethroscopy showed a papillary tumour on a 1-cm stalk at the base of the bladder with two tumours in the posterior urethra. The tumours were resected at cystoscopy and Thiotepa was instilled for one hour. Histological examination showed that the bladder tumour was a well differentiated transitional cell carcinoma with no evidence of invasion. At repeat cystoscopy on 28 May the resected area was oedematous but there was no evidence of recurrence.

Discussion

The peak prevalence of bladder carcinoma is in the sixth decade of life and the condition is rare in patients less than 40 years of age. The tumour is epidemiologically linked with schistosomiasis, with contact with 2-napthylamine and with rubber anti-oxidants, and with smoking more than 15 cigarettes a day.[4] We were unable to find any published reports relating venereal disease to bladder carcinoma but it is interesting that Kaplan *et al*,[5] in a retrospective study, reviewed 232 cases of primary carcinoma of the urethra and found a history of venereal disease in 37%. Ashworth[6] reviewed 756 cases of bladder carcinoma and found simultaneous urethral tumours in 14 (1·8%) patients. The associated urethral tumours were most often found in the posterior urethra, especially if the bladder tumour was near the internal meatus, as it was in the present case.

Our patient had lived in Jamaica until the age of 4 when he came to live in Birmingham. In Birmingham he worked as a chrome-plater but moved to London in the mid-1970s to work as a motor mechanic. His history about smoking was vague, but he insisted he smoked less than 15 cigarettes a day. He denied previous venereal disease and his serological tests for syphilis gave negative results.

It is possible that this patient's tumour was either occupational in origin or was related to his smoking, but since his first attack of haematuria occurred at the early age of 20 this seems unlikely. No obvious definite predisposing factor could be found and indeed it was thought initially that his haematuria was due to his gonococcal infection. This view was reinforced by the rapid disappearance of the haematuria after treatment with penicillin. The pre-existing lesions in the urethra and the bladder presumably allowed the gonococcus to behave more aggressively and to present as a haemorrhagic cystitis. There was no blood at the meatus when the anterior urethra was massaged and hence probably little, if any, bleeding from the anterior urethra.

It would thus seem advisable to carry out full urinary tract investigations in all cases of gonorrhoea which present with florid haematuria.

The authors wish to thank Mr R W Lloyd-Davies for permission to report this case and also Dudley Road Hospital, Birmingham, for allowing access to the patient's previous notes.

References

1. Boyd JT, Csonka GW, Oates JK. Epidemiology of non-specific urethritis. *Br J Vener Dis* 1958; **34:** 40-3.
2. Amarasuriya KL. Haematuria presenting in outpatients attending a department of genitourinary medicine. *Br J Vener Dis* 1979; **55:** 214-7.
3. Barlow D, Phillips I. Gonorrhoea in women. diagnostic, clinical, and laboratory aspects. *Lancet* 1978; **1:** 761-4.
4. Hendry WF, Bloom HJG. Urothelial neoplasia. In: Hendry WF, ed. *Recent Advances in Urology 2*. Edinburgh, London and New York: Churchill Livingstone, 1976: 245-92.
5. Kaplan GW, Bulkley GJ, Grayhack JT. Carcinoma of the male urethra. *J Urol* 1967; **98:** 365-71.
6. Ashworth A. Papillomatosis of the urethra. *Br J Urol* 1956; **28:** 3-11.

Br J Vener Dis 1980;56:107-10.

Vaginal pH and microflora related to yeast infections and treatment

S M DRAKE,* B A EVANS,† AND A GERKEN‡

From the Departments of †Genitourinary Medicine and ‡Medical Microbiology, Charing Cross (West London) Hospital, London

SUMMARY The relationship between vaginal pH, microflora, and yeast infection was investigated in 93 women randomly treated with either nystatin or miconazole pessaries and cream for two weeks. The vaginal pH was measured in a control group of 48 women. In the study group, 37 patients defaulted, 39 were cured, and 17 required treatment during the six-month follow-up period. In both study and control groups before and after treatment the mean vaginal pH was in the range of 4·3-4·6. Lactobacilli were plentiful in 78 (91%) out of 86 patients and shows that lactobacilli and yeasts commonly coexist. The influence of other organisms appeared to be negligible. The trial showed that nystatin and miconazole were equally effective in the treatment of vaginal yeast infection and that the broad-spectrum activity of miconazole offered no advantage in this condition.

Introduction

Several previous reports have been published concerning the relationship between vaginal yeasts, other microflora, and their effects on pH. Peeters and his co-workers[1] found a higher pH (in the lateral fornix) in patients with clinical signs or symptoms of candidal vaginitis than in controls, but not all patients had the diagnosis confirmed by culture. They also concluded that there was no correlation between pH, symptoms, and the number of colonies of pathogens, but an inverse relationship between the presence of candida and lactobacilli was found. Cohen[2] demonstrated a lower pH in the posterior fornix in women with clinical vaginitis than in controls. Both studies included some pregnant women and women with other vaginal pathogens and are therefore not directly comparable, but they do indicate conflicting results concerning vaginal pH and the presence of yeasts.

In the present study, our aim was to monitor the changes in pH values in a group of women with yeast infection in response to treatment. We also examined changes in pH values in relation to the menstrual cycle and the use of oral contraceptives. At the same time we assessed the vaginal flora and its changes in response to therapy with the standard antifungal agent, nystatin, and a newer broad-spectrum imidazole derivative, miconazole, with activity against a wide range of fungi and some Gram-positive bacteria.

Patients and methods

STUDY GROUP

The study group consisted of 93 women attending the Department of Genitourinary Medicine at the West London Hospital who had symptoms of vaginitis, or whose contacts had balanitis, and who had yeast cells or hyphae detected on a Gram-stained smear. Details of last sexual intercourse, last menstrual period, method of contraception, recent treatment, parity, contact's symptoms, and relevant past history were all recorded. Symptoms and signs, together with the pH value and the results of smears and cultures for *Trichomonas vaginalis, Neisseria gonorrhoeae,* and yeasts, were noted. Samples were taken on each occasion for culture of lactobacilli and other vaginal micro-organisms.

After diagnosis by microscopy the patients were treated randomly with either nystatin two pessaries at night for two weeks, with cream twice daily, or miconazole one pessary at night for two weeks, with cream twice daily. These treatments were prepackaged in plain coded envelopes so that the prescriber did not know which treatment was given. The patients were asked to attend monthly for three months for follow up and thereafter if symptoms returned.

Address for reprints: Dr B A Evans, Department of Genitourinary Medicine, West London Hospital, Hammersmith Road, London W6 7DQ

Received for publication 20 March 1979

CONTROL GROUP

The control group was composed of a random sample of 48 patients of similar age, country of origin, and contraceptive practice attending the same department and who were free of genital infection.

MEASUREMENT OF pH

Vaginal pH was measured using a Corning-Eel digital pH meter with a combination electrode with semimicro tip (requiring only 0·3 ml of fluid). The electrodes were calibrated for each measurement using two buffers, pH 7·0 and pH 4·01, between which they were rinsed thoroughly with deionised water. Various techniques for obtaining the measurements were tried, but the most stable reproduceable pH readings were obtained by inserting the electrode directly into the vagina to a distance of approximately 4 cm. By this method the pH reading stabilised within 5-10 seconds. In each case the average of three readings to two decimal places was obtained. The electrodes were washed with deionised water between recordings. After each patient had been tested the electrode was cleaned under running tap water and placed in 2% glutaraldehyde solution for at least 30 minutes; the electrodes were then immersed in pH 7 buffer until required again. All recordings of buffer were made at room temperature and the meter was adjusted to 37°C for vaginal readings.

MICROBIOLOGY

To exclude the principal pathogens the following investigations were carried out:

N gonorrhoeae

Gram-stained smears from the cervix and urethra were examined for typical Gram-negative intracellular diplococci. Samples were inoculated directly on to "split" plates containing a modified Thayer-Martin medium in one half and the same base without antibiotics in the other. The plates were incubated immediately for 48 hours at 37°C in candle-extinction jars. *N gonorrhoeae* was identified on culture by oxidase reaction and microscopical appearance in Gram-stained films. Confirmation was by immunofluorescent staining.[3]

T vaginalis

Direct examination of a wet preparation of vaginal secretion was accompanied by culture in Feinberg-Whittington medium and examined after 48 hours' incubation at 37°C.

Yeasts

A Gram-stained smear of vaginal material was examined and cultures made on Sabouraud's agar. Cultures were incubated for 48 hours at 37°C and any yeasts isolated were identified by (1) germ-tube production; (2) chlamydospore formation on corn meal agar; (3) typical colonial appearance on tetrazolium agar; and (4) sugar fermentation tests.

Other micro-organisms

A high vaginal swab was taken and sent to the laboratory in Stuart's transport medium. The following culture plates were inoculated: 2×5% horse blood agar; MacConkey agar; Rogosa agar (Difco); and mycoplasma agar (base and supplement, BBL). The cultures were incubated aerobically for 48 hours at 37°C except one blood agar plate, which was placed in an anaerobic environment. The mycoplasma plates were incubated in 10% CO_2 for five days.

Ureaplasmas and chlamydia were not looked for. *Corynebacterium vaginale* was not specifically identified. The term, Gram-variable cocco-bacilli, was used to describe organisms of similar morphology.

Organisms were identified according to standard methods.[4] Lactobacilli were detected by typical growth on Rogosa agar.[5]

An additional smear of vaginal material was Gram-stained and examined later for yeasts in all those cases in which the initial positive smear result was not supported by a positive yeast culture result. Lactobacilli were also looked for in all cases with negative culture results on Rogosa agar.

ANALYSIS OF DATA

The response to treatment was analysed by the χ^2 test with Yates's correction.

Results

STUDY GROUP

The 93 women were aged between 15 and 49 years (mean 24·5 years). A past history of yeast infection was obtained in 50 (54%). In 25 (27%) a penile irritation or rash was present in a sexual partner. Eighty-nine women (97%) were symptomatic and 88 (95%) had a vulval itch or discharge. Oral contraception was used by 52 (56%) women. Presentation for treatment at the clinic in relation to the menstrual cycle is shown in Table I.

TABLE I *Diagnosis in relation to menstrual cycle*

	Week of menstrual cycle				
	1	*2*	*3*	*4*	*Total*
No of patients with yeast infection*	13	25	25	26	89

*Two patients were post-menopausal and two had amenorrhoea

The mean pH of the study group was 4·6 (Table II). No patient was menstruating at the time of diagnosis. Three patients had trichomoniasis and one gonorrhoea; there was only one woman whose partner had non-specific urethritis and she also had trichomonal infection both before and after initial treatment for yeast infection. In the study group, neither the outcome of treatment nor the drug given was related to, or had an effect on, vaginal pH (Table III).

TABLE II *pH values for study and control group of patients*

Patient group	*Total No of patients*	*Range of pH* 3·5-3·9	4·0-4·4	4·5-4·9	5·0-5·4	5·5-5·9	6·0-6·4	6·5-6·9	*Mean*
Study	93	9	39	29	7	6	2	1	4·6
Control	48	4	23	7	7	6	1		4·6

TABLE III *pH values of study group related to treatment*

Study group	*Mean pH*	*No*	*%*
Initial pH	4·6	93	100
Cured patients' initial pH	4·6	39	42
Cured patients after treatment	4·3	39	42
Relapsing patients' initial pH	4·5	17	18
Relapsing patients at relapse	4·4	13	14
Nystatin-treated patients at 1 month	4·1	16	17
Miconazole-treated patients at 1 month	4·3	12	13

CONTROL GROUP

The 48 control patients were aged 18-45 years (mean 25·4 years). The mean pH was 4·6 (Table II). No patient was menstruating at the time of examination. None was infected with yeasts, trichomonads, or gonococci; three patients were sexual contacts of men with non-specific urethritis. No variation in pH was found throughout the menstrual cycle and there was no relationship with oral contraception (Table IV).

TREATMENT (STUDY GROUP)

The outcome of treatment is shown in Table V. Vaginal infection recurred within six months in 11 (12%) patients, who were treated with nystatin and in 6 (6%) patients, who were treated with miconazole (χ^2_1 0·213, P=0·5). At the first follow-up visit, two weeks after completion of treatment, only three patients had relapsed, two treated with nystatin and one treated with miconazole.

TABLE IV *pH values of control group related to week of menstrual cycle and contraception*

Menstrual cycle (week) and contraception	*Mean pH*	*No*	*%*
1	4·7	11	23
2	4·5	11	23
3	4·6	12	25
4	4·7	14	29
Contraceptive pill	4·6	31	65
Others	4·6	17	35

TABLE V *Outcome of treatment*

Treatment	*No of patients* *Defaulted**	*Cured†*	*Relapsed (months)* *1*	*2*	*3*	*4*	*Total*
Nystatin	15	21	2	5	2	2	47
Miconazole	22	18	1	4	1	0	46
Total	37	39	3	9	3	2	93

*No follow-up test
†At least one negative culture result

MICROBIOLOGY (STUDY GROUP)

The yeasts were identified as *Candida albicans* in 91 patients. One patient was colonised by *Torulopsis glabrata* and one by *Candida parapsilosis*.

Samples for extended microbiological studies were received from 86 patients at the initial visit. Follow-up samples were received from 52 patients—37 on one occasion, 11 on two occasions, and four at three follow-up visits.

The relationship in pre-treatment samples between vaginal pH values, presence of lactobacilli, and other organisms is shown in Table VI. In addition to lactobacilli, the other organisms isolated were:

Bacteroides species
Bacillus coli
β-haemolytic streptococci
Diphtheroids
Faecal streptococci
Gram-variable cocco-bacilli
Klebsiella species
Mycoplasma species
Non-haemolytic streptococci
Proteus species
Staphylococcus aureus
Staphylococcus epidermidis

TABLE VI *Relationship between pre-treatment vaginal pH values and presence of lactobacilli and other bacteria on culture*

	Mean (range) pH values *4·2 (3·9-4·7)*	*4·7 (3·7-6·5)*	*4·9 (4·2-5·8)*
No of patients	23	55	8
Culture results			
Lactobacilli	Abundant	Moderate	Absent or very scanty
Other organisms	Scanty	Abundant	Abundant

The distribution throughout the samples was variable. No predominant organism was found in specimens with scanty or no lactobacilli, but follow-up specimens from this group showed the recurrence of lactobacilli in three of four patients investigated after treatment.

No notable change in microflora was found after treatment in the other groups examined.

Discussion

The question of the relationship between vaginal pH values and morbidity due to *C albicans* and other infections has produced conflicting reports with regard to the benefit of a low vaginal pH. Our work has indicated no relationship between vaginal pH over the range observed in 93 patients and 48 controls (pH 3·6-6·5) and vaginal morbidity due to yeast infection. Thus the protective effect of vaginal acidity does not appear to be confined to a critical range above or below which there is increased susceptibility to vaginal infection.

A relationship between the numbers of lactobacilli and vaginal acidity, however, is clearly shown and is consistent with the physiological role of the organism in maintaining a low pH in the vagina. Moreover, it appears that the part played by other organisms in the vagina rarely assumes importance, even when the pH value is at the higher end of the range observed. Increasing dominance of any organism normally considered commensal may lead to an inflammatory vaginal reaction, but this did not occur in our experience among patients with candidal infection.

Treatment with both drugs was highly effective in the short term, with mycological cure in all but three who returned for follow up at one month (a cure rate of 91·3% with nystatin and of 94·7% with miconazole). Comparison of cure rates subsequently is increasingly invalidated by an inability to distinguish treatment failure from reinfection with further yeasts.

We would like to thank Janssen Pharmaceutical Ltd for kindly providing the pH meter and all the materials used in this study. Our thanks are also due to the Sister and staff of the Martha Clinic, the staff of the Medical Microbiology Department, West London Hospital, and Dr B Partridge and Miss M Denny of the Medical Microbiology Department, Charing Cross Hospital Medical School, London.

References

1. Peeters F, Snauwaert R, Segers J, Van Cutsem J, Amery W. Observations on candidal vaginitis: vaginal pH, microbiology and cytology. *Am J Obstet Gynecol* 1972; **112:** 80-6.
2. Cohen L. Influence of pH on vaginal discharges. *Br J Vener Dis* 1969; **45:** 241-6.
3. Beck A, Fluker JL, Platt DJ. *Neisseria meningitidis* in urogenital infection. *Br J Vener Dis* 1974; **50:** 367-9.
4. Cowan ST, Steel KJ. *Manual for the Identification of Medical Bacteria.* 2nd ed. Cambridge University Press: London, 1974.
5. Thomas Mair EM. Isolation and identification of lactobacilli from the genital tract. *PHLS Monograph 5* HMSO: London, 1974.

Br J Vener Dis 1980;**56**:111-4.

Proctitis and herpes simplex virus in homosexual men

D GOLDMEIER
From the Whitechapel Clinic, the London Hospital

SUMMARY In a study of the prevalence of rectal infection with herpes simplex virus (HSV) in a group of homosexual men the magnified appearance of the rectal mucosa correlated with isolation of the virus. HSV was isolated from rectal material from five of 77 men with proctitis of unknown cause but from none of 44 control patients without proctitis; two of four men with HSV proctitis were asymptomatic. Thus, the magnified rectal mucosal image, showing severe congestion, haemorrhage, and pus, appears to be a sensitive indicator of the presence of HSV proctitis.

Introduction

Herpes simplex virus (HSV) is a cause of urethritis in less than 1% of men with nongonococcal urethritis.[1] Although studies indicate that HSV is a cause of proctitis in homosexual men[2 3] there are no reports of the prevalence of this infection. The present study was undertaken to estimate this in two groups of patients—those with and those without proctitis. A correlation between the magnified rectal mucosal appearance, as seen through the operating microscope, and the presence of HSV in rectal material was also attempted.

Patients and methods

All homosexual men who attended the Whitechapel Clinic at the London Hospital or the Diagnostic Clinic at Moorfields Eye Hospital between 1 July 1974 and 30 November 1975 were included in the study. Only those men who admitted to passive penorectal intercourse in the previous year were included. Full sexual and general medical histories were taken, including details of the most recent date of passive intercourse and the number of such contacts in the four weeks before initial attendance. Patients who admitted to taking antibiotics within the month before attending hospital were excluded from the trial. Details of the survey were explained to the patient, whose verbal consent was then obtained.

DIAGNOSTIC CRITERIA FOR PROCTITIS

Proctitis was defined when one or more of the following changes were visualised through an operating microscope using × 16 and × 25 magnification: (a) minimal to moderate mucosal congestion (Fig 1); (b) severe mucosal congestion (Fig 2); (c) haemorrhagic appearance (Fig 3); and (d) the presence of pus-like material in the rectum which contained at least 10 leucocytes per microscopic field (× 1000).

Patients with known ulcerative proctitis, Crohn's disease of the rectum, gonococcal or chlamydial proctitis, or an early syphilitic lesion in the anorectal area were excluded from the study.

CLINICAL EVALUATION

A full general examination was made initially. The rectal mucosa was then viewed through a metal proctoscope wth the patient lying in the left lateral position. Subsequently, a magnified image of the mucosa (× 6, × 10, × 16, and × 25) was obtained by means of a Zeiss operating microscope (Carl Zeiss Ltd). Photographs of the area were taken using an Alpha Reflex 9F camera (Pignons, Switzerland) attached to the microscope. Any pus-like material found by such examination was removed with a sterile bacteriological loop, smeared on to a glass slide, fixed by heat, and stained by Gram's method.

Rectal material was obtained for culture for HSV by thoroughly rubbing a cotton-tipped wooden swab (Johnson and Johnson Ltd) over the rectal area in view. This swab was placed in HSV transport medium (medium 199 with Hanks balanced salt solution, bovine plasma albumin 0·5%, gentamycin 50 mg/ml, and mycostatin 50 mg/ml) and stored at 4°C for less than 12 hours. To exclude other causes of proctitis, rectal material was obtained for culture for *N gonorrhoeae* and *Chlamydia trachomatis* as previously described.[4]

Repeated darkfield examinations were made on

Address for reprints: Dr D Goldmeier, Department of Genitourinary Medicine, University College Hospital, London WC1

Received for publication 13 August 1979

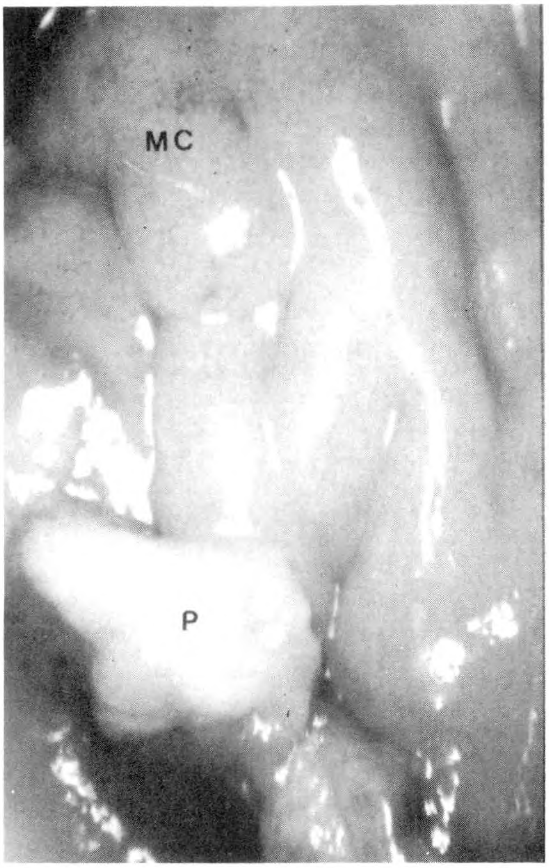


FIG 1 *Photomicrograph showing minimal-to-moderate mucosal congestion (MC) with pus (P) (× 16)*

serum from anal ulcers, if present, to exclude syphilis. The throat was then visualised and tonsillar and faucial material transferred on a cotton-tipped wooden swab for culture for HSV.

LABORATORY INVESTIGATIONS

After transportation rectal and throat material were transferred on to a monolayer of human embryonic lung cells for culture. These cells were incubated and inspected daily for seven days to exclude the presence of specific cytopathic effects of HSV. Where possible, the virus was typed according to the cellular morphology of the culture.

The method of culture of rectal material for *N gonorrhoeae* and *C trachomatis* has been described in detail elsewhere.[4]

Blood was taken at the first visit for serological tests for syphilis (automated reagin test and Reiter protein complement fixation test), and for storage of serum for later testing, using the HSV complement fixation test (HSVCFT) where rectal cultures had given positive results. Where herpetic proctitis was diagnosed, serum was tested again some weeks after the initial attendance using the HSVCFT.

STATISTICAL AND DEMOGRAPHIC DETAILS

Demographic details were obtained from all patients. Snedecor's *t* test was used to compare mean values obtained in test and control groups.

Results

Seventy-seven patients had proctitis of unknown cause. HSV was isolated from five of these but from none of the 44 control patients who did not have proctitis. The demographic details of the two groups, which showed no obvious bias, are given in Table I. The difference between the means of the number of passive peno-rectal encounters of patients in the two groups during the four weeks before initial attendance (proctitis 2·25, no proctitis 1·96) was not significantly different ($P>0·05$).

Diagnostic details for the patients with proctitis are given in Table II. The clinical pictures and laboratory

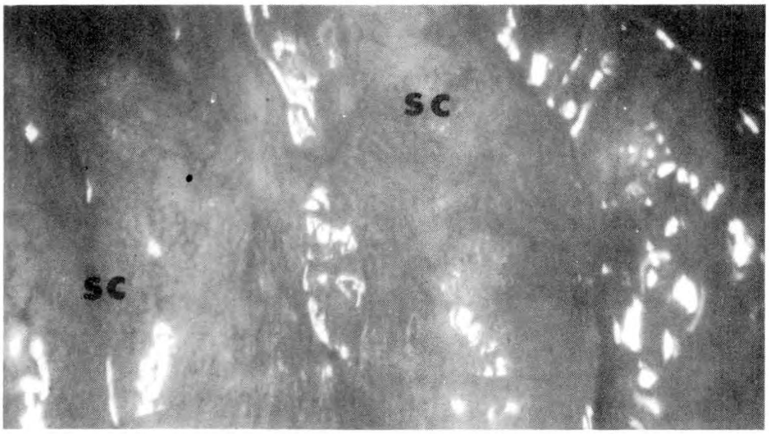


FIG 2 *Photomicrograph showing severe mucosal congestion (SC) (×25)*

TABLE I *Demographic details of study and control groups*

Demographic details	*With proctitis (n = 77)*	*Without proctitis (n = 44)*
Mean age (years)	32·4	31·3
Ethnic group		
Caucasian	73	42
Negroid	3	2
Indian	1	0
Occupation		
Clerical	16	11
Manual/unskilled	21	10
Manual/skilled	15	13
Professional	18	8
Others	7	2
Total	77	44

details of the five patients with proctitis from whom HSV was obtained from rectal material are given in Tables III and IV. HSV was not grown from throat material of any of the five patients.

Discussion

HSV was grown from rectal material from five (6%) men with proctitis. No throat isolates were obtained in these men, so that the virus presumably did not reach the rectum by a transintestinal route. This study thus estimates the prevalence of HSV in homosexual men with proctitis. HSV is known to be excreted from the cervix and vulva from asymptomatic women with previous clinically apparent HSV infections.[5] Patients 4 and 5 (Table III) appeared to be asymptomatic excretors of HSV, although in the case of one of them examination with the naked eye indicated a mild proctitis. A more accurate indication of the frequency of periods of infectivity in this population would be obtained by following a cohort of such men with a known history of excreting HSV in rectal material over a period of time.

This work was also undertaken to attempt to correlate the clinical findings with positive culture results from rectal material. In none of the 44 patients who showed no evidence of proctitis was HSV cultured. Because only 6% of those with proctitis were infected with HSV it would seem impracticable to screen all homosexual men with proctitis for HSV. However, two of the four men with severe mucosal congestion and haemorrhagic change, noted by means of the operating microscope, were found to be excreting HSV. Similarly, two (14%) of 14 men with severe mucosal congestion were excreting the virus. Hence, the use of the operating microscope seems to be a practicable screening technique for herpetic proctitis.

The author is grateful to the Medical Research Council for a grant to carry out this work (grant No. G 973/515/C) and to Irene Prentice for her excellent illustration. My grateful thanks are given to Dr E M C Dunlop for his encouragement and help with the comments at all stages of this work; to Dr P Rodin for his invaluable advice; to the Virology Department of the London Hospital Medical College for HSV cultures; and to Mr S Goldsmith and his staff of the Whitechapel Clinic for their clinical assistance.

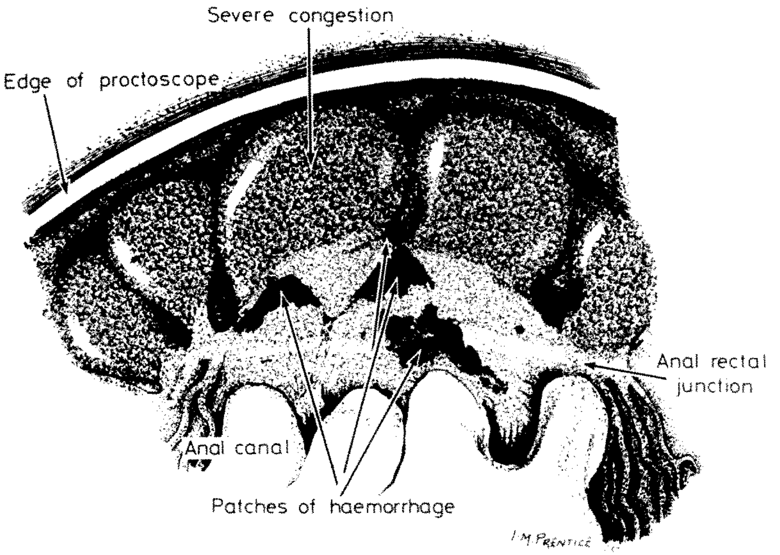


FIG 3 *Illustration of anorectal area with proctitis and haemorrhagic mucosa (× 16)*

TABLE II *Microscopical appearance (× 25) of rectal mucosa in 77 men with proctitis (figures in parentheses indicate mucosal changes visible to the naked eye)*

	Mucosal surface change only	*Pus and mucosal change*	*Pus-like material only*	*Total*
Minimal-to-moderate congestion	18 (5)	10 (3§)	0	28 (8)
Severe congestion	3 (3‡)	11 (11†)	0	14 (14)
Severe congestion and haemorrhage	0 (0)	4 (4*)	0	4 (4)
Pus-like material	0	0	31 (8)	31 (8)
Total	21 (8)	25 (18)	31 (8)	77 (34)

* Two of the four patients culture-positive for HSV
† One of 11 patients culture-positive for HSV
‡ One of three patients culture-positive for HSV
§ One of three patients culture-positive for HSV

TABLE III *Clinical details of the five patients with proctitis and rectal HSV*

Patient No	*Last passive intercourse (days)*	*No of contacts in previous 4 weeks*	*Previous HSV infection*	*Symptoms*	*Anal lesions*	*Mucosal appearance*	
						Naked eye	*× 25 magnification*
1	9	1	Oral "cold sore" 4 months before	Anorectal soreness/loose motions	Ulcers	Congestion	H SC P
2	9	2		Anorectal discharge	Ulcers unnoticed by patient	Congestion and pus	H SC P
3	1	1		Anorectal soreness and discharge/ loose motions	Ulcers	Congestion	SC P
4	7	4		Asymptomatic	None	Congestion	SC
5	4	2		Asymptomatic	None	Normal	MC P

H = Haemorrhagic areas
MC = Minimal-to-moderate congestion
SC = Severe congestion
P = Pus

TABLE IV *Laboratory results of the five patients with proctitis and rectal HSV*

Patient No	*HSV type*	*HSVCFT titre*		*Time taken for mucosa to return to normal (weeks)*
		Initial	*Convalescent**	
1	2	1/10	1/160 (2)	7
2	1	1/10	1/20 (2)	4
3	1	1/10	1/80 (2)	3
4	Untyped	1/20	1/10 (2)	3
5	Untyped	1/80	Unavailable†	Unknown

* Time (in months) from initial visit given in brackets
† Patient defaulted

References

1. King A, Nicol C. *Venereal Diseases*. 3rd ed. London. Baillière and Tindall. 1975.
2. Goldmeier D, Bateman JRM, and Rodin P. Urinary retention and intestinal obstruction associated with anorectal herpes simplex virus infection. *Br Med J* 1975; 1: 425-6.
3. Jacobs E. Anal infections caused by herpes simplex virus. *Dis Colon Rectum* 1976; 19: 151-7.
4. Goldmeier D. A study of non-specific proctitis in homosexual men. University of London M D Thesis 1976: 70-8.
5. Rattray MC, Corey L, Reeves WC, Vontver LA, Holmes KK. Recurrent genital herpes among women—symptomatic vs asymptomatic viral shedding. *Br J Vener Dis* 1978; 54: 262-5.

Br J Vener Dis 1980;**56**:115-6.

First African Regional Conference on Sexually Transmitted Diseases

Inauguration of the African Branch of the International Union against the Venereal Diseases and Treponematoses

The First African Regional Conference on Sexually Transmitted Diseases and the establishment of the African Branch of the International Union against the Venereal Diseases and Treponematoses (IUVDT) took place at the conference centre of the University of Ibadan, Ibadan, Nigeria, from 21 to 26 August 1979. The conference had been carefully planned by Professor A O Osoba and his colleagues and the arrangements and the management of the meetings were of the highest standards.

The conference was well attended and over 70 members were present in addition to many paramedical participants. Twenty-two nations were represented and there were three members from the USA, two from France, and one each from Belguim, Brazil, and the United Kingdom. The IUVDT was represented by the President, Dr R D Catterall.

Opening and guest speakers

The conference was opened by Professor S O Olayide, the acting vice-chancellor of the University of Ibadan, who welcomed the participants to the university campus. The very high prevalence of sexually transmitted diseases (STD) throughout the world and particularly in Africa was stressed by Dr M O Ogunlana, honourable commissioner for health, Oyo State, Nigeria. Professor C O Enwonwu, director of the National Institute of Medical Research of Nigeria, discussed the importance of accurate statistics and the scope for research in the subject, which had been neglected in the past.

The guest speakers included Dr R D Catterall (London), who described the constitution, history, and present activities of the Union, Professor Corintho Santos, director of the regional office for Latin American, Dr Paul J Wiesner, director, Venereal Disease Control Division, Center for Disease Control, Atlanta, Georgia, USA, who spoke on the challenge of STD control in developing countries, and Dr A Siboulet, director of the Institut Alfred Fournier, Paris, who discussed epidemiological treatment of patients with STD and some new and current aspects of gonococci.

Later Mr E O Nkposong, consultant urologist, University College Hospital, Ibadan, described post-inflammatory causes of infertility and the whole day's proceedings were characterised by lively and well informed discussion.

The meetings

On the following day Dr R D Catterall (London) spoke on the world situation of the sexually transmitted diseases and Dr R Widiwersky, World Health Organisation medical officer, Bangui, Central African Republic, described the management of gonorrhoea in Africa. Papers by Dr Corintho Santos on STD in Latin America and on STD in Africa by Professor I Diop-Mar, Clinique des Maladies Infectieuses, University of Dakar, followed.

A whole session was devoted to Togamycin (spectinomycin) in the management of gonorrhoea in Africa and a lively discussion followed. It was suggested that this injectable drug should be used in treatment failures with penicillin and when β-lactamase strains were isolated.

Papers on STD in Africa were contributed by Dr E E Omer (Khartoum), Dr R V Ratnam (Lusaka), Professor O Ogunbi (Lagos), Professor A Meheus (Swaziland), Dr K O Alausa (Ibadan), Dr R M Rafols (Lesotho), and Dr T T A Elemile (Ibadan).

Dr K O Alausa (Ibadan) discussed vulvo-vaginitis in children in Ibadan and Dr Anne Retel-Laurentin (Paris) the medical and social complications of STD in Haute-Volta.

Dr C S S Bello (Zaria), presented a paper on the results of an investigation into the knowledge of STD among practising doctors in the northern states of Nigeria and there were papers on chlamydial infection and trichomoniasis.

Dr M Gedebou (Addis Ababa) and Professor H Nsanze (Nairobi) painted a sombre picture of the spread of antibiotic resistance in their countries.

Contact-tracing in developing countries was vividly described by Mrs I O Ajayi (Ibadan), and Professor A Z Meheus (Antwerp) discussed gonorrhoea control based on simplified health technology.

Business meeting of the African Union

A business meeting of the African Union against the Venereal Diseases and Treponematoses was held on the last day of the conference and was chaired by Dr R D Catterall, President of the IUVDT.

The following resolutions were passed and are to be widely circulated:

(1) The African Union against the Venereal Diseases and Treponematoses calls the attention of African governments to the high incidence and serious consequences of sexually transmitted diseases in Africa and hereby expresses its concern at this situation.

(2) The Union therefore calls upon African governments to try to rectify this situation by providing increased resources and facilities for free diagnosis and treatment.

(3) The Union recommends that all African governments institute a campaign to enlighten health personnel and the general public, especially through youth organisations and institutes, about the hazards of sexually transmitted diseases using all available means of communication.

(4) The Union calls on all institutions responsible for training health personnel to review and up-date their sexually transmitted diseases training programmes and to encourage and intensify research into sexually transmitted diseases.

The following were elected officers of the African Union for a period of two years: President, Professor I Diop-Mar (Senegal); Vice Presidents, Dr A Lama (Guinea) and Professor H Nsanze (Kenya); Secretary-general, Professor A O Osoba (Nigeria); Assistant Secretary-generals, Dr B Ndiaye (Senegal) and Professor V L Ongom (Uganda); Treasurer, Professor A B O Oyediran (Nigeria); Councillors, Professor A D W Acholonu (Nigeria), Professor M Gedebou (Ethiopia) Dr E E Omer (Sudan), and Professor V L Ongum (Uganda).

The proposed constitution was unanimously adopted, subject to the French version being approved by the French-speaking officers of the Union.

The next African regional conference will be held in 1981 in Dakar, Senegal, at a date to be decided.

Social programme

Several excellent films were shown and there was an extensive programme, which included cocktail parties, a luncheon, and a dinner. On the final day there was an excursion to Ile-Ife to visit the museum and the University of Ife.

The conference was widely reported on television, radio, and in the press and provoked a lively interest in the medical profession and among the lay public.

Comment

The formation of the African Union against the Venereal Diseases and Treponematoses as the African Branch of the IUVDT is an important occasion in African medical history. The conference indicated that there is a high prevalence of infection in all African countries and that in most areas the facilities are totally inadequate or non-existent. There is an increasing awareness and a greater interest in the subject among doctors and paramedical workers.

Many of the most up-to-date clinics for sexually transmitted diseases in Africa are staffed and directed by members of the department of microbiology. In some teaching hospitals the equipment and premises have improved but in most clinics either there is no equipment at all or what is available is hopelessly inadequate. In many clinics very large numbers of patients are seen daily and the problem is growing rapidly.

The First African Regional Conference was a great success and had been carefully prepared and planned by Professor A O Osoba. It could be the start of a new era in Africa for the diagnosis, treatment, and management of patients with sexually transmitted diseases and might eventually lead to their control throughout the continent.

R D Catterall

Br J Vener Dis 1980;**56**:117.

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Management of non-specific urethritis in men

Sir,

Dr Evans's criticism[1] of one aspect of our paper[2] is well taken but was made slightly out of context. Taking the whole sample first, we had already found that "of the 143 patients who were retreated 66 (46·2%) admitted to unprotected sexual intercourse compared with 103 (27·5% of 375) who did not require retreatment. This difference was significant (χ^2_1 15·607; P<0·001)." The "sub-sample" of 112 provided further confirmation of this point as well as evidence (not acceptable to Dr Evans) as regards the effect of treatment of female sexual contacts on the retreatment rate of men. We did not claim these 112 men to be a statistical sample of the whole nor did we choose our couples. We simply included all couples who could be identified. We then made comparisons within this so-called sub-sample between two categories—that is, those requiring retreatment and those not requiring retreatment—and found that the difference was significantly related to sexual intercourse. We observed that sexual intercourse did not make any difference to the outcome of treatment with the single-dose doxycycline regimen. But the pattern of treatment failure with all other regimens in this respect was different (Table 3[2]). We, therefore, consider the comparison between the sub-sample of 112 and the total sample of 562 to be irrelevant, especially in so far as it does not invalidate the comparisons within the sub-sample already mentioned.

As regards Dr Evans's suggestion of delayed failures, Dr Evans certainly knows that the longer the follow up the greater the chances of further sexual intercourse taking place and hence reinfection occurring. (Indeed as many as 71% of the patients retreated after five weeks in this study were associated with sexual intercourse.) If a venereologist does not recognise this fact then some contacts will remain untreated, thus contributing to a further spread of this sexually transmitted disease.

Finally, most of the retreatments took place between two and six weeks after completion of the initial regimen.

Yours faithfully,

O P Arya
C D Alergant
E H Annels
P B Carey
A K Ghosh
A D Goddard

Royal Liverpool Hospital,
Liverpool L7 8XP

References

1. Evans B. Letter: Management of non-specific urethritis in men. *Br J Vener Dis* 1979; **55**: 305.
2. Arya OP, Alergant CD, Annels EH, Carey PB, Ghosh AK, Goddard AD. Management of non-specific urethritis in men: Evaluation of six treatment regimens and effect of other factors including alcohol and sexual intercourse. *Br J Vener Dis* 1978; **54**: 414-21.

TO THE EDITOR, *British Journal of Venereal Diseases*

Future planning of venereological services

Sir,

Regional health authorities are considering the future of their venereology services. In the Northern Regional Strategic Plan (1979-88) it is noted that in 1977 the mean number of new patients per session in their clinics was 3·5 and the mean total patients per session was 12·2 compared with 7·0 and 23·0 respectively for England as a whole. From these figures the authors of the Plan concluded that "the workload figures do not suggest that additional clinic provision should be considered at present."

These workload figures were obtained from the SH3 returns, the forms submitted monthly by clinics stating the numbers of sessions, new patients, and total attendances.

Study of the clinic time-tables in 1977, which have since been rationalised, indicates several factors which contributed to the lower than average number of new patients and total attendances per session. Throughout the region the mean duration of a session was just under two hours (1·95). In one sea-port clinic there was only one two-hour session per week, and this was for women only. At another clinic, male and female patients were seen at separate times, six of their sessions per week were of one hour's duration, five of one and a half hours, and one of two hours' duration, but the clinic reported having 12 sessions per week for the 15½ hours it was open. Yet another clinic reported that it held 26 sessions per week, when in fact it was only open for 22. In addition, in 1977, 13 dual (where one doctor saw male and female patients) sessions per week were carried out by one doctor, although they were reported as 26 sessions. This grossly underestimated these doctors' workloads, and they were only paid for 13 sessions.

Happily it appears that only the numbers of new patients are being used to judge workload, although a good case could be made out for considering the number of conditions diagnosed and treated. Total attendances would not be a good guide to workload because, between clinics, there are such wide variations in the number of post-treatment attendances required for any one condition, and whereas some clinics record an attendance for each condition, others record just one attendance irrespective of the number of conditions that have been treated.

If workload, as reported in the SH3 returns, is to be the criterion on which the future of the venereology service is to be planned, then physicians in charge of clinics should ensure that sessions of short duration are not reported as though they were full notional ones and that when one doctor carries out a dual session it is recorded as one and not two sessions.

Yours faithfully,

C B S Schofield

Newcastle General Hospital,
Westgate Road,
Newcastle upon Tyne, NE4 6BE

Br J Vener Dis 1980;**56**:118.

Book review

The Management of Sexually Transmitted Diseases: A guide for the general practitioner. R R Willcox, 1979. Pp 79, tables 9. WHO Regional Office for Europe, Copenhagen (price not known).

The foreword to this admirable little book starts by pointing out that in Europe, as in other parts of the world, most patients suffering from sexually transmitted diseases are treated in general practice by physicians with inadequate training in the management of such diseases. As a result they may pay insufficient attention to the need for laboratory investigations in making a correct diagnosis and for contact-tracing. This book has been prepared to fill this need on the recommendation of the World Health Organisation and is published by the WHO Regional Office for Europe. While it is primarily designed for general practitioners, it will also be useful for aspiring specialists and will start them on the correct lines for managing their patients.

The common clinical presentations of the sexually transmitted diseases are clearly and concisely described, and the appropriate laboratory investigations are discussed in some detail. Practical advice is given on treatment, contact-tracing, and follow up.

There is a very useful section summarising the management of patients presenting with specific problems such as urethral discharge. In this, the options for management are listed in declining order of efficiency. It is suggested that the general practitioner should assess his or her own practice and capabilities and seek to improve them if necessary.

The final section summarises the drugs used in the treatment of the sexually transmitted diseases.

This book is a most useful addition to the range of concise publications on this subject, and fulfils the resolution of the 31st World Health Assembly, which requested the director-general of the WHO to "draw up, disseminate, and up-date as required guide lines on the control of sexually transmitted diseases." The book reflects Dr Willcox's wide clinical experience, his teaching experience, and his capabilities as a writer on this subject. It will be valuable in all countries where English is understood, and it is published in French and in Russian. I hope the crispness and clarity of the text will be maintained in these translations.

R N Thin

Br J Vener Dis 1980;**56:**119-124

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea
(Clinical; microbiology; therapy)
Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases
Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (clinical and therapy)

Epidemiology of infectious syphilis at a tertiary hospital
LM DRUSIN, B TOPFOLSTEIN, AND E LEVYZOMBEK (Department of Epidemiology, New York Hospital, USA). *Arch Intern Med* 1979;**139:**901-4.

Hepatic ultrastructure in secondary syphilis
SEH BROOKS, B HANCHARD, S TERRY, AND JJ ANDRETSCH (University of the West Indies, Jamaica). *Arch Pathol Lab Med* 1979; **103:**451-5.

Membranous glomerulonephritis in congenital syphilis
A LOSITO, E BUCCIARELLI, F MASSI-BENEDETTI, AND M LATO (Institute of Paediatrics, Perugia, Italy). *Clin Nephrol* 1979; **12:**32-6.

Syphilis (serology and biological false-positive phenomenon)

Diagnostic evaluation of syphilis during pregnancy
JE JONES AND RE HARRIS (San Antonio, USA). *Obstet Gynecol* 1979;**54:**611-3.

Utility of the FTA-ABS test of cerebrospinal fluid in the diagnosis of neurosyphilis
T McGEENEY, F YOUNT, DR HINTHORN, AND C LIU (University of Kansas, USA). *Sex Transm Dis* 1979;**6:**195-8.

Syphilis (pathology and experimental)

Endemic syphilis: passive transfer of resistance with serum and cells in hamsters
RF SCHELL, JK CHAN, AND JL LEFROCK (Albany Medical Center Hospital, New York, USA). *J Infect Dis* 1979; **140:** 378-83.

Endemic syphilis was studied in hamsters to determine whether serum or cells from animals rendered resistant to infection with *Treponema pallidum* (strain Bosnia) could confer resistance to syphilis in normal animals. The hamsters received either immune or normal serum at intervals for four weeks. Three days after the first injection they were challenged. A third group of animals received no serum and were similarly challenged with *T pallidum.* Lesions developed in all animals except those vaccinated with immune serum. The lymph nodes of the resistant animals contained a large number of treponemes but this was less than in the other two groups. Passive transfer of resistance with spleen cells using either normal or immunised hamsters showed after challenge that those animals having received cells from immunised hamsters did not develop syphilitic lesions. Treatment of the immune spleen cells with antilymphocytic serum and complement abolished the ability to transfer resistance. This appears to be the first direct evidence that thymus-derived cells are involved in resistance to syphilis.

G W Csonka

Suppression of lymphocyte response to concanavalin-A by mucopolysaccharide material from *Treponema pallidum*-infected rabbits
RF BEY, RG JOHNSON, AND TJ FITZGERALD (University of Minnesota, USA). *Infect Immun* 1979;**26:**64-9.

The effect of reducing and other agents on the motility of *Treponema pallidum* in an acellular culture medium
WSK CHALMERS, AND D TAYLOR-ROBINSON (Clinical Research Centre, Harrow, UK). *J Gen Microbiol* 1979;**114:**443-7.

Gonorrhoea (clinical)

Gonococcal vulvovaginitis in prepubertal girls
TC FREWEN AND RM BANNATYNE (Hospital for Sick Children, Toronto, Canada). *Clin Pediatr* 1979;**18:**491-3.

Gonococcal pelvic inflammatory disease; economic impact in New York, Medicaid 1975
RT PULICE, AND DO LYMAN (Californian Department of Health, USA). *N Y State J Med* 1979;**79:**1728-9.

Gonococcal endocarditis in the antibiotic era
DB COOKE, D ARENSBERG, JM FELNER, D RIMLAND, AND LM LESSER (Emory University School of Medicine, Atlanta, Georgia, USA). *Arch Intern Med* 1979;**139:**1247-50.

Before the introduction of penicillin up to 26% of cases of infectious endocarditis were due to *Neisseria gonorrhoeae*. Recently this condition, which had almost disappeared, has started to increase again (three cases are described) and should be suspected in any patient under the age of 30 and without pre-existing heart disease. It most commonly affects the aortic wall and may show a long latent period after the primary infection. As many as six blood cultures may be necessary to establish the aetiology. If the patient is not allergic to penicillin large doses should be given for 4-6 weeks. If the patient is allergic to penicillin desensitisation followed by penicillin therapy is preferable to other antibiotics. In two of the present cases aortic valve replacement became necessary.

G W Csonka

Gonorrhoea (microbiology)

In-vitro susceptibility of penicillinase-producing *Neisseria gonorrhoeae* to a select group of antimicrobial agents, alone and in combination (letter)
TT YOSHIAWA AND SA SHIBATA (University of California, USA). *J Antimicrob Chemother* 1979;**5**:618-9.

In a letter, the authors report the in-vitro susceptibility of 10 strains of β-lactamase-producing *Neisseria gonorrhoeae* to various antimicrobial agents. The strains were all susceptible to spectinomycin, trimethoprim /sulphamethoxazole (TMP/SMZ) in combination, cefazolin, amikacin, and, with one exception, to tetracycline. All strains were highly resistant to penicillin G, ampicillin, and, with one exception, streptomycin. A synergistic effect of the TMP/SMZ combination in a ratio of 1 : 19 reduced inhibitory concentrations to 0·31 and 5·9 mg/l respectively compared with >5 and >95 mg/l separately. Previous studies by the same author showed that a single dose of nine tablets of commercial TMP/SMZ (ratio 80 mg : 400 mg) will achieve blood concentrations of 2·16 and 31·7 mg/l respectively at 24 hours, well above the inhibitory concentrations effective against β-lactamase gonococci used in the study. Oral treatment on a single-dose basis may therefore be possible for these organisms.

Brian Evans

Evaluation of five serologic tests for antibody to *Neisseria gonorrhoeae*
S TOSHACH, J COULL, S SIGURDSON, S DUBLENKO, J GROCHOLSKI, AND L LINAREZ (Provincial Laboratory of Public Health, Edmonton, Canada). *Sex Transm Dis* 1979;**6**:214-7.

Production of β-lactamase by a strain of *Neisseria gonorrhoeae* when cultured in the presence of ethidium bromide (letter)
S HAFIZ, TO ODUGBEMI, I GEARY, AND MG McENTEGART (University of Sheffield, UK). *Lancet* 1979;ii:844.

β-lactamase-inhibitors—a novel approach (editorial)
Lancet 1979;ii:565-6.

Protection by monospecific gonococcal antisera of the chick embryo challenged with *Neisseria gonorrhoeae*
JN ROBERTSON (Southampton General Hospital, UK). *J Med Microbiol* 1979; **12**:283-9.

Sensitivity of *Neisseria gonorrhoeae* to partially purified R-type pyocines and a possible approach to epidemiological typing
CC BLACKWELL, H YOUNG, AND I ANDERSON (University of Edinburgh, UK). *J Med Microbiol* 1979;**12**:321-35.

Comparison of a slide coagglutination technique with the Minitek system for confirmation of *Neisseria gonorrhoeae*
KD HAMPTON, RA STALLINGS, AND BL WASILAUSKAS (Wake Forest University, Winston Salem, N Carolina, USA). *J Clin Microbiol* 1979;**10**:290-2.

Antibodies to *Neisseria gonorrhoeae*: a study of the urethral exudates of 232 men
A McMILLAN, G McNEILLAGE, AND H YOUNG (University of Edinburgh, UK). *J Infect Dis* 1979;**140**:89-95.

Comparison of the penicillin-binding proteins of different strains of *Neisseria gonorrhoeae*
RD NOLAN AND JF HILDEBRANDT (ICI Pharmaceuticals, Macclesfield, UK). *Antimicrob Agents Chemother* 1979; **16**:336-40.

Assessment of attachment of *Neisseria gonorrhoeae* to HeLa cells by double radiolabelling
ER GUBISH, ML MACE, SM STEINER, AND RP WILLIAMS (Baylor University, Texas, USA). *Infect Immun* 1979;**25**:1043-50.

Co-transformation of temperature sensitivity and nutritional markers in *Neisseria gonorrhoeae*
VL STEINBERG, AC FENTON, EH BAKER, AND ID GOLDBERG (University of Kansas, USA). *J Bacteriol* 1979;**140**:206-12.

Evaluation of a culture procedure for identification of *Neisseria gonorrhoeae*
L HELLGREN, G JOHANNISON, AND J VINCENT (University of Trondheim, Norway). *Ann Clin Res* 1979;**11**:98-100.

Effects of low ampicillin concentrations on penicillin-sensitive and β-lactamase-producing strains of *Neisseria gonorrhoeae*
T ELMROS, S HOLM, E KJELLBERG, S NORMARK, AND B WINBLAD (University of Umea, Sweden). *J Antimicrob Chemother* 1979;**5**:555-61.

Screening by culture for the detection of gonorrhoea in women
H YOUNG, AB HARRIS, D URQUHART, AND DHH ROBERTSON (University of Edinburgh, UK). *Scott Med J* 1979;**24**:302-6.

Killing of *Neisseria gonorrhoeae* by human polymorphonuclear neutrophil granule extracts
RF REST (College of Medicine, Tucson, Arizona, USA). *Infect Immun* 1979; **25**:574-9.

Phenotypically determined resistance of *Neisseria gonorrhoeae* to normal human serum: environmental factors in subcutaneous chambers in guinea pigs
M GOLDNER, CW PENN, SC SANYAL, DR VEALE, AND H SMITH (University of Birmingham, UK). *J Gen Microbiol* 1979; **114**:169-77.

Variations in surface protein composition associated with virulence properties in opacity types of *Neisseria gonorrhoeae*
PR LAMBDEN, JE HECKELS, LT JAMES, AND PJ WATT (University of Southampton, UK). *J Gen Microbiol* 1979; **114**:305-12.

The ecology of gonococcal plasmids
M ROBERTS, P PIOT, AND S FALKOW (University of Washington, USA). *J Gen Microbiol* 1979; **114**:491-4.

Gonorrhoea (therapy)

Cefoxitin treatment of penicillinase-producing *Neisseria gonorrhoeae*
SW BERG, ME KILPATRICK, WO HARRISON, AND JA McCUTCHAN (US Navy, San Diego, USA). *N Engl J Med* 1979;**301**:509-11.

Cefoxitin is a semisynthetic cephamycin unaffected by β-lactamase. A trial is reported in which cefoxitin 2 G i.m. was compared with procaine penicillin G 4·8 megaunits i.m. in a randomised sequence. One hundred and seven men with culture-positive urethral gonorrhoea attended for follow up. The 54 patients treated with cefoxitin were all cured, although four had no follow-up culture performed. However, of the 53 treated with penicillin only 34 were cured, and of the 19 who failed 17 had β-lactamase-producing organisms; in the penicillin-treated group 22 patients had β-lactamase-positive organisms and in the cefoxitin-treated group, 21. Five patients who received cefoxitin complained of pain at the injection site despite reconstitution in 4 ml 0·5% lignocaine. Cefoxitin is claimed to be an effective alternative to spectinomycin for uncomplicated gonorrhoea caused by β-lactamase producing strains.

Brian Evans

Successful treatment with cefaclor of gonococcal urethritis in men

VA SPAGNA, RL PERKINS, AND RB PRIOR (Ohio State University, USA). *Sex Transm Dis* 1979; **6**: 211-3.

Non-specific genital infection

Response to treatment of chlamydial infection of the cervix (letter)

J HUNTER, IW SMITH, A MACAULEY, AND JF PEUTHERER (University of Edinburgh, UK). *Lancet* 1979; ii: 848-9.

In a short report the response to treatment of chlamydial infection of the uterine cervix was examined by comparing post-treatment isolates in 59 women treated with oxytetracycline or doxycycline for one week with the same drugs for 14-21 days.

Chlamydia was isolated from nine patients after treatment, five having taken tetracyclines for one week and four for 2-3 weeks. Reinfection was considered a problem in seven women, as two had resumed intercourse with regular untreated partners and five, initially negative, had positive results on subsequent examination, again after resuming intercourse with regular untreated partners.

Although the study was small it showed that a week of tetracycline is as effective in eradicating chlamydia as a three-week course and re-emphasised the importance of investigating and treating regular sexual partners with sub-group A chlamydial genital infection.

R S Pattman

Genital mycoplasma infection: intrauterine infection: pathologic study of the fetus and placenta

MR DISCHE, PA QUINN, E CZEGLEDY-NAGY, AND JM STURGESS (Le Bonheur Childrens' Hospital, Memphis, USA). *Am J Clin Pathol* 1979; **72**: 167-74.

Mycoplasmal infection was present in the fetuses from three spontaneous abortions and in one neonate born in the second trimester. Gross examination revealed in most cases a severely infected placenta and membranes with a fetus of normal appearance. The fetal infection presumably followed placental involvement and appeared to have been acquired shortly before delivery. Genital mycoplasmas, *Ureaplasma urealyticum,* or *Mycoplasma hominis* were isolated from the placentas and the fetal tissues, and genital tracts of the mothers. Isolation of the mycoplasmas from the liver indicated that bloodstream dissemination of these organisms occurred in the fetus. In the fetus the pathological changes were variable. Lesions were identified in the lung by scanning electron microscopy of the bronchial tree in two cases and were accompanied by interstititial pneumonia. An abnormally dilated left ventricle suggesting cardiomyopathy was observed in one case.

Authors' summary

Delayed hypersensitivity to *Chlamydia trachomatis:* cause of chronic prostatitis? (letter)

RC BALLARD, C BLOCK, JH KOORNHOF, AND B HAITAS (University of Witwatersrand, Johannesburg, South Africa). *Lancet* 1979; ii: 1305-6.

The authors of this case report describe the clinical course of a patient with the clinical features of chronic prostatitis. A 36-year-old man presented with a three-year history of pain in the perineum, lower back, and both testes; exacerbation and remission of these symptoms were noted during that period. The prostate was enlarged and tender, and an epididymitis was also noted. Although *Chlamydia trachomatis* was not isolated from the urethra of the patient nor from the cervix of his wife, chlamydial antibodies were detected in the sera from both. That the patient had developed delayed hypersensitivity to chlamydial antigens was demonstrated by a skin test. The patient's clinical condition improved when he was treated with a 5½-month course of erythromycin.

A McMillan

Survival of *Ureaplasma urealyticum* on different kinds of swabs

SA POULIN, RB KUNDSIN, AND HW HORNE (Peter Brent Brigham Hospital, Boston, USA). *J Clin Microbiol* 1979; **10**: 601-3.

The survival of *Ureaplasma urealyticum* on five different types of swab routinely used for obtaining clinical specimens was investigated with 16 ureaplasma-positive urine specimens as inoculum: each type of swab was tested with two (minimum) to four (maximum) urine samples. Swabs charged with ureaplasmas were rolled on A7 agar medium and then placed in Ford liquid medium. With certain types of swab, colonies were obtained on A7 agar although the corresponding Ford broth cultures gave negative results. Neither cotton-tip or pledget, nor rayon-tip, nor calcium-alginate tip, nor aluminium shaft, nor polyester applicator was by itself inhibitory to the ureaplasmas. However, the wooden applicator stick exerted an inhibitory effect sufficient to prevent a colour change in all ureaplasma-positive urine specimens. Ureaplasmas remained viable, since negative broths when subcultured after 18 hours produced colonies on A7 agar. After storage at room temperature for longer than one hour survival of ureaplasmas was poor on all swabs.

In a clinical trial cervical swabs were taken from 54 patients with a cotton-tipped wooden applicator swab and a rayon-tipped swab wrapped around a polyester applicator; both A7 agar and Ford liquid broth were inoculated with each swab. The latter swabs gave a positive result in 25 patients, both on A7 medium and in Ford broth, but the wooden applicator swabs, although positive with A7 medium in 25 patients, yielded only 11 positive broth culture results.

H Young

Rosamicin, a macrolide with in-vitro activity against *Ureaplasma urealyticum*

AC HILL AND G SUTTON (Medical Research Council, Carshalton, Surrey, UK). *J Antibiot* 1979; **32**: 915-8.

Genital *Chlamydia trachomatis* infections with cervical atypia

J PAAVONEN, E VESTERINEN, B MEYER, P SAIKKU, J SUNI, E PUROLA, AND E SAKSELA (University of Helsinki, Finland). *Obstet Gynecol* 1979; **54**: 289-91.

Preparation of antigens for microimmunofluorescence testing for antichlamydial antibodies (letter)

IT NISBET, DM GRAHAM, KM NG, AND PA LUNT (University of Melbourne, Australia). *Lancet* 1979; ii: 859.

Immunofluorescence testing for chlamydial antibodies (letter)
SJ RICHMOND (Public Health Laboratory, Bristol, UK). *Lancet* 1979;ii:1016.

Immunofluorescence testing for chlamydial antibodies (letter)
T FORSEY AND S DAROUGAR (Institute of Ophthalmology, London, UK). *Lancet* 1979;ii:1016.

Comparison of various McCoy cell treatment procedures used for detection of *Chlamydia trachomatis*
RT EVANS AND D TAYLOR-ROBINSON (Clinical Research Centre, Harrow, UK). *J Clin Microbiol* 1979;**10:**198-201.

Formalinized *Chlamydia trachomatis* organisms as antigens in the microimmunofluorescence test
S-P WANG, C-C KUO, AND JT GRAYSTON (University of Washington, Seattle, USA). *J Clin Microbiol* 1979;**10:**259-61.

Genital tract-to-eye infection: tissue culture of *Chlamydia trachomatis*
JG MARKHAM (National Health Institute, Wellington, New Zealand). *N Z Med J* 1979;**90:**186-7.

Reticulate bodies as single antigen in *Chlamydia trachomatis* serology with microimmunofluorescence
EC YONG, JS CHINN, HD CALDWELL, AND C-C KUO (University of Washington, Seattle, USA). *J Clin Microbiol* 1979;**10:**343-5.

Wheat germ agglutinin blockage of chlamydia attachment sites: antagonism by N-acetyl-D-glucose
NJ LEVY (University of Chicago, USA). *Infect Immun* 1979;**25:**946-53.

Demonstration of chlamydial endotoxin-like activity
VJ LEWIS, WL THACKER, AND SH MITCHELL (Centre for Disease Control, Atlanta, Georgia, USA). *J Gen Microbiol* 1979;**114:**215-6.

Reiter's disease

The clinical features and HLA associations of reactive arthritis with nongonococcal urethritis
AC KEAT, RN MAINI, GD PEGRUM, AND JT SCOTT (Charing Cross Hospital, London, UK). *Q J Med* 1979;**48:**323-42.

Fifty-seven patients with arthritis associated with nongonococcal genital infection have been studied. Synovitis characteristically affected one or a few joints, especially the knee, ankle, or metatarsophalangeal joints, and was accompanied by tenosynovitis and enthesopathies, each in about one-third of the patients. A quarter of the patients had ocular, cutaneous, or mucus membrane lesions (Reiter's syndrome). Although six patients developed a chronic or relapsing course, the average duration of the acute episode in most was three to five months. Available evidence strongly suggests that infection following sexual intercourse, usually but not always with a new partner, was instrumental in the initiation of the disease. We have suggested the term "sexually acquired reactive arthritis (SARA)" to emphasise the mode of acquisition of the disease, and note that similar syndromes are seen associated with gut infection. We consider that usage of the term Reiter's syndrome is correctly applied to only those cases which exhibit the characteristic triad of urethritis, arthritis, and conjunctivitis with or without other cutaneous and mucus membrane lesions. Thirty-six of the 54 patients who were HLA-typed (67%) possessed the antigen HLA-B27. Of 30, who presented directly to a rheumatology unit, 25 (83%) were HLA-B27-positive. The other 24 patients initially attended a venereology clinic and only 11 (46%) of these bore the antigen. This appears to reflect the severity of the disease, HLA-B27-positive patients having a significantly longer duration of disease symptoms and a higher frequency of extra-articular manifestations than those lacking this antigen.

Authors' summary

Ankylosing spondylitis and chlamydial infection in apparently healthy HLA-B27 blood donors
M ALCALAY, B AMOR, F HAIDER, D ALCALAY, J ORFILA, J TANZER, AND D BONTOUX (Hôpital Cochin, Paris, France). *J Rheumatol* 1979;**6:**439-46.

HLA-B27 in patients with seronegative spondarthritides
AN MALAVIYA, NK MEHRA, G ADHAR, K JINDAL, S BHARGAWA, RK BATTA, MC VAIDYA, AND B SANKARAN (Safdarjung Hospital, New Dehli, India). *J Rheumatol* 1979; **6:**413-6.

Reiter's syndrome (editorial)
Lancet 1979;ii:567.

Reiter's syndrome after salmonella infection—occurrence in HLA-B27-positive brothers
MB JONES, PW SMITH, AND RW OLNHAUSEN (University of Nebraska, USA). *Arthritis Rheum* 1979;**22:**1141.

Trichomoniasis

Lack of evidence for cancer due to use of metronidazole
CM BEARD, KL NOLLER, WM O'FALLON, LT KURLAND, AND MB DOCKERTY (Mayo Clinic, Rochester, USA). *N Engl J Med* 1979; **301:**519-21.

Candidosis

Requirement of heat-labile opsonins for maximal phagocytosis of *Candida albicans*
A FERRANTE AND YH THONG (University of Adelaide, Australia). *Sabouraudia* 1979; **17:**293-7.

Using a radiometric method, the requirement of heat-labile opsonins for phagocytosis was investigated. It was shown that these opsonins were required for maximal phagocytosis of two serologically distinct strains and 10 clinical isolates of *C albicans*. There was a 50% loss in the phagocytic potential of the test system when serum which had previously been heated to 56°C for 30 minutes was used in place of untreated serum.

A McMillan

Biochemical identification of clinically important yeasts
J DE LUVOIS, A MULHALL, AND R HURLEY (Queen Charlotte's Maternity Hospital, London, UK). *J Clin Pathol* 1979; **32:**715-8.

Assimilation of protocatechuic acid and p-hydroxybenzoic acid as an aid to the laboratory identification of *Candida parapsilosis* and other medically important yeasts
BH COOPER AND GA LAND (Baylor University, Dallas, Texas, USA). *J Clin Microbiol* 1979; **10:**343-5.

Abortion associated with intrauterine infection by *Candida albicans* (case report)
R BUCHANAN, MK SWORN, AND AD NOBLE (Royal Hampshire City Hospital, UK). *Br J Obstet Gynaecol* 1979;**86:**741-4.

Purification of a mannan from *Candida albicans* which activates serum complement
TL RAY, A HANSON, LF RAY, AND KD WEPPER (University of Oregon, USA). *J Invest Dermatol* 1979;**73:**269-74.

Genital herpes

Association of herpes simplex virus (HSV) with cervical cancer by lymphocyte reactivity with HSV-1 and HSV-2 antigens
JW SMITH, JE TORRES, AND ND HOLMQVIST (Louisiana State University, USA). *Am J Epidemiol* 1979;**110:**141-4.

Demonstration of exogenous genital reinfection with herpes simplex virus type 2 by restriction endonuclease fingerprinting of viral DNA
TG BUCHMAN, B ROIZMAN, AND AJ NAHMIAS (University of Chicago, USA). *J Infect Dis* 1979;**140:**295-304.

Comparative prevalence of subclinical cytomegalovirus and herpes simplex virus infections in the genital and urinary tracts of low-income, urban women
GE KNOX, RF PASS, DW REYNOLDS, S STAGNO, AND CA ALFORD (University of Alabama, USA). *J Infect Dis* 1979;**140:**419-22.

Subclinical herpes simplex genitalis infection in the perinatal period
N TEJANI, SW KLEIN, AND M KAPLAN (Nassau City Medical Center, New York, USA). *Am J Obstet Gynecol* 1979;**135:**547.

Other sexually transmitted diseases

Aetiological association of virus hepatitis and primary carcinoma of the liver in Germany
B EHRLICH *Dtsch Med Wochenschrift* 1979;**104:**1316.

Aetiological association of virus hepatitis B and carcinoma of the liver has been repeatedly suggested. In the present series of 25 patients (19 men) with confirmed primary carcinoma of the liver a high proportion were either B antigen- or antibody-positive and 54·5% had had intimate contact with B hepatitis in the past. Similarly, a high proportion of cases of cirrhosis of the liver, which was also present in 22 of the group with primary carcinoma of the liver, showed this close association with B hepatitis. A control group of 548 normal individuals showed a significantly lower presence of hepatitis B antigen or antibody and only 7·4% had close contact with active hepatitis B. These results support other reports that hepatitis B virus infection is an important factor in cirrhosis of the liver, which may progress to carcinoma of the liver. (If this association is definitely confirmed, Australia antigen B hepatitis becomes one of the most sinister conditions so commonly encountered in clinics dealing with sexually transmitted diseases).

G W Csonka

Treatment of infestation with *Phthirus pubis*: comparative efficacies of synergized pyrethrins and benzene hexachloride
JH NEWSON, HB HORTON, AND HW BAKER (University of Oregon, USA). *Sex Transm Dis* 1979;**6:**206-10.

A new non-prescription pediculicidal liquid, essentially 0·3 pyrethrins synergised by 3% piperonyl butoxide, was compared with 1% γ-benzene hexachloride, only available on prescription in the USA, for the treatment of infestation with *Phthirus pubis*.

Thirty adult patients with *P pubis* infestation were divided into two groups matched for age, sex, and race and randomly treated with either a single 10-minute application of the synergized-pyrethrin liquid or a single 12-hour application of γ-benzene hexachloride lotion. The response to treatment was assessed by examining the pubic hair and water used to rinse the pubic area for lice and nits immediately after the prescribed time period and observing the patients after a week.

All adult lice and nymphs were dead at the conclusion of a single treatment in both groups and after a week all patients were free from lice, nymphs, and viable nits. No adverse side effects occurred after application of either medication.

It was concluded that the synergized-pyrethrin preparation is an advance, as it appears to be free of toxicity problems associated with chlorinated hydrocarbons and in the USA is available without prescription, enabling self-treatment without the embarrassment of seeking medical advice.

R S Pattman

Chronic hepatitis B infection in male homosexuals
P SKINHOJ, G HOYBYE, B HENTZER, V FABER, AND H PONSEN (Rigshosp, Copenhagen, Denmark). *J Clin Pathol* 1979;**32:**783-5.

Ten cases of hepatitis B virus infection were identified among asymptomatic male homosexuals. These patients shared a number of characteristics: a subclinical origin and course of infection; persistence of HBsAg for periods exceeding six to 25 months; persistent GPT elevation of 2-5 times upper normal limit; and morphological changes in the liver with portal and parenchymal inflammation (chronic persistent hepatitis, six cases; non-specific reactive hepatitis, two cases; cirrhosis and acute hepatitis with signs of chronicity, one case each). HBeAg was found in six cases, anti-HBe in none.

These results indicate that screening for hepatitis B should be performed whenever these individuals seek medical attention in order to detect asymptomatic chronic liver diseases and to detect these silent vectors of an infection that is at present increasing among homosexuals.

Authors' summary

***Neisseria meningitidis* urethritis**
MA MILLER, P MILLIKIN, PS GRIFFIN, RA SEXTON, AND M YOUSUF (Peoria School of Medicine, Illinois, USA). *JAMA* 1979; **242:**1656-7.

***Neisseria meningitidis*. Probable pathogen in two related cases of urethritis, epididymitis, and acute pelvic inflammatory disease**
DC WILLIAM, YM FELMAN, AND MC CORSARO (Bureau of Venereal Disease Control, New York, USA). *JAMA* 1979;**242:**1653-4.

Condylomata acuminata as a sign of sexual abuse in children
J SEIDEL, J ZONANA, AND E TOTTEN (University of California, USA). *J Pediatr* 1979;**95:**553-4.

Virus-specific and anticellular antibodies in molluscum contagiosum
PV SHIRODARIA, RS MATTHEWS, AND M SAMUEL (Queen's University, Belfast, N Ireland). *Br J Dermatol* 1979;**101:**133-40.

Primary extragenital disseminated cutaneous donovanosis
VN SEHGAL, NL SHARMA, NC BHARGAVA, M NAYAR, AND M CHANDRA (Panchwati, India). *Br J Dermatol* 1979;**101:**353-6.

Public health and social aspects

Screening for gonorrhea and syphilis in gay bathhouses in Denver and Los Angeles
HI MERINO, FN JUDSON, D BENNET, AND TR SCHAFFNIT (Disease Control Service, Denver, Colorado, USA). *Public Health Rep* 1979;**94:**376-8.

Effects of socioeconomic status on incidences of three sexually transmitted diseases
WE MORTON, HB HORTON, AND HW BAKER (University of Oregon, USA). *Sex Transm Dis* 1979;**6**:206-10.

Social stratification, sexual behavior and the sexually transmitted diseases (editorial)
WW DARROW (Centre for Disease Control, Atlanta, Georgia, USA). *Sex Transm Dis* 1979;**6**:228-30.

Miscellaneous

Genital bacteriology—comparative study of premenopausal women with postmenopausal women
NG OSBORNE, RC WRIGHT, AND L GRUBIN (University of Connecticut, USA). *Am J Obstet Gynecol* 1979;**135**:195-8.

In view of reports that premenopausal women are at a higher risk than postmenopausal women of postoperative infection following vaginal hysterectomy a study was designed to compare the genital flora of premenopausal women less than 36 years of age with that of postmenopausal women over 55 years of age. All of the women admitted to the study attended a private clinic and were healthy and free of any genital symptoms. Bacteriological investigation included culture for aerobes, anaerobes, and mycoplasmas. No group of organisms was found to be significantly predominant in any set of patients and no statistically significant differences were found in the number of species of microorganisms isolated from the cervix and vagina of premenopausal and postmenopausal women ($P>0 \cdot 5$). These results are interpreted to suggest that there are factors in addition to bacteria which influence the rate of postoperative infection. Other factors which have been proposed to account for the differences in the risk of postoperative infection in premenopausal and postmenopausal patients are discussed, as is the possible role of the host defence system. (A study of the microbial flora of premenopausal and postmenopausal patients before undergoing hysterectomy would be of interest.)

H Young

Quantitative microbiology of human vulva
R ALY, MB BRITZ, AND HI MAIBACK (University of California, USA). *Br J Dermatol* 1979;**101**:445-8.

The microbial flora of the vulva is described and compared with the flora of the forearm by utilising the detergent scrub method. Microbial counts were higher on the vulva ($2 \cdot 8 \times 10^6/cm^2$) than on the forearm ($6 \cdot 4 \times 10^2/cm^2$). Lipophilic diphtheroids, coagulase-negative staphylococci, micrococci, non-lipophilic diphtheroids, and lactobacilli formed the dominant flora of the vulva. Streptococci, Gram-negative rods, and yeasts were also noted. The highest incidence of *Staphylococcus aureus* was noted on the vulva (67%) followed by the perianal area (30%), nose (30%), and the forearm (11%).

Authors' summary

Do contraceptives influence the incidence of acute pelvic inflammatory disease in women with gonorrhoea
G RYDEN, L FAHRAEUS, L MOLIN, AND K AHMAN (Linkoping University Hospital, Sweden). *Contraception* 1979;**20**:149-55.

The incidence of pelvic inflammatory disease among contraceptive and non-contraceptive users was studied in 672 patients with gonorrhoea. Pelvic inflammatory disease was diagnosed in 87 and confirmed by laparoscopy. There was a significantly lower incidence of pelvic inflammatory disease in the group using hormonal contraception (8·8%) than in those using copper intrauterine devices (IUDs) (23·5%) ($P<0 \cdot 001$) and in those using neither hormones nor IUDs (15·1%) ($P<0 \cdot 02$).

There were 20 cases of pelvic inflammatory disease in the 85 patients using IUDs. Six of these became ill within three weeks of insertion of the IUD; it was not known whether or not these patients had had genitourinary gonorrhoea before insertion. If these patients are excluded, the risk of pelvic inflammatory disease for women with an IUD for longer than three weeks was not greater than for the non-IUD and non-hormonal users, but it was still greater than for the group using hormonal contraceptives ($P<0 \cdot 05$).

J M Harvey

Inhibition of *Haemophilus vaginalis (Corynebacterium vaginale)* by metronidazole, tetracycline and ampicillin
ED RALPH, TW AUSTIN, FLM PATTISON, AND BC SCHIEVEN (University of Western Ontario, Canada). *Sex Transm Dis* 1979;**6**:199-202.

The etiology and pathogenesis of pelvic inflammatory disease (editorial)
FG CUNNINGHAM (University of Texas, USA). *Sex Transm Dis* 1979;**6**:221-3.

The etiology of acute pelvic inflammatory disease (editorial)
DA ESCHENBACH AND KK HOLMES (University of Washington, Seattle, USA). *Sex Transm Dis* 1979;**6**:224-7.

Lactobacillus for vulvovaginitis (letter)
B SANDLER (Manchester, UK). *Lancet* 1979;ii:791-2

Prostatosis, prostatitis or pelvic floor tension myalgia
JW SEGURA, JL OPITZ, AND LF GREENE. *J Urol* 1979;**122**:168-9.

Use of vaginal cleansing kit in non-specific vaginitis
HSK SINGHA (University of Southampton, UK). *Practitioner* 1979;**223**:403-4.

Behcet's syndrome (editorial)
DG JAMES (Royal Northern Hospital, London, UK). *N Engl J Med* 1979;**301**:431-2.

Relationship between sexual intercourse and urinary tract infection in women attending a clinic for sexually transmitted diseases
MC KELSEY, MG MEAD, RN GRUNEBERG, AND JD ORIEL (University College Hospital, London, UK). *J Med Microbiol* 1979;**12**:511-2.

Sacroiliitis and salpingitis
E SZANTO AND K HAGENFELDT (Karolinska Hospital, Stockholm, Sweden). *Scand J Rheumatol* 1979;**8**:129-35.

FORTY-EIGHT HOUR PATIENCE

One tablet nightly for three nights means that your patient's patience only needs to last 48 hours. That's all it takes to clear vaginal candidiasis – twice as conveniently as the old One Two Free.

Canesten®
clotrimazole

New 200mg vaginal tablet now only one a night for three nights

Presentations Canesten is available either as 200 mg vaginal tablets in packs of three with applicator or as 100 mg vaginal tablets in packs of six with applicator. Canesten Duopak contains six 100 mg vaginal tablets with applicator plus 20 g cream containing 1.0% clotrimazole. **Dosage** *Vaginal Tablets 100mg* Two vaginal tablets should be inserted at night for three consecutive days. Alternatively, one vaginal tablet daily for six days. *Vaginal Tablets 200mg* One vaginal tablet should be inserted at night for three consecutive days. *Duopak* Vaginal Tablets 100 mg as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to prevent re-infection. **Side effects** Rarely patients may experience transient local mild burning or irritation. **Basic NHS Costs** 6 x 100 mg vaginal tablets with applicator £2.13; 3 x 200 mg vaginal tablets with applicator £1.99; Duopak £3.47. **Product Licence Numbers** Vaginal tablets 100 mg, PL 0010/0015; vaginal tablets 200 mg, PL 0010/0072; Duopak, PL 0010/0016 & PL 0010/0015. Further information is available from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. ® Registered Trade Mark of Bayer Germany.

The combination of simple language and straightforward line drawings has made the

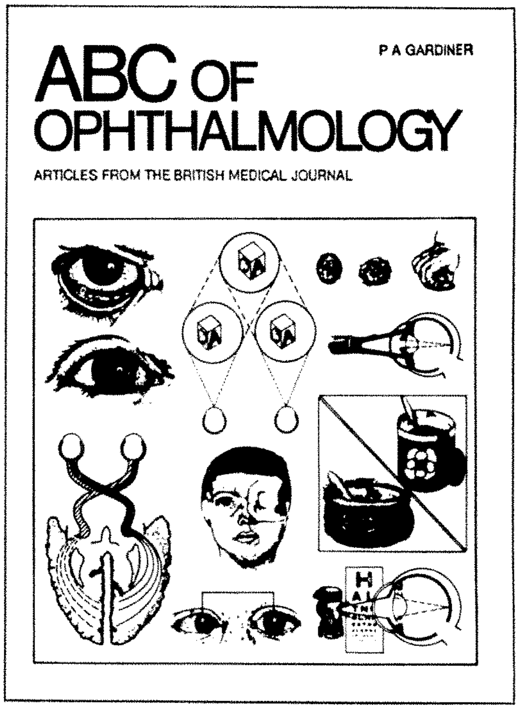
ABC OF OPHTHALMOLOGY

one of the most popular series of articles in the Medical Practice section of the British Medical Journal.

All the common problems are covered - visual defects, squints, glaucoma and cataracts - together with detailed instruction on examination, diagnosis and management.

The articles have now been collected into book form - in exactly the same format as they appeared in the BMJ - price: Inland £2.50; Overseas US$6.25 (Inland £2.00; Overseas US$5.00 for BMA members).

Order your copy now
From: The Publisher,
British Medical Journal,
BMA House,
Tavistock Square,
London WC1H 9JR
or any leading bookseller



P A GARDINER

ABC OF OPHTHALMOLOGY

ARTICLES FROM THE BRITISH MEDICAL JOURNAL

Sexually Transmitted Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $25.00 outside the United States ($22.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $28.00 in the United States and $30.00 in all other countries.

Michael F. Rein, M.D.
American Venereal Disease Association
Box 385
University of Virginia School of Medicine
Charlottesville, Virginia 22901 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $25.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases*.

☐ I wish to enter a Non-Member or institutional subscription ($28.00 U.S., all other countries $30.00).
(Mail non-member subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105)

Name ______________________________

____

Address ______________________________

City ______________ State Country ______________ Zip ______________

British Journal of Venereal Diseases April 1980 Vol. 56 No. 2

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Some aspects of venereal diseases in Greenland
ELLIS FROM (Denmark) *page* 65

Immunoglobulin-bearing lymphoid cells in primary syphilis
J D BOS, F HAMERLINCK, AND R H CORMANE (the Netherlands) *page* 69

T lymphoid cells in primary syphilis
J D BOS, F HAMERLINCK, AND R H CORMANE (the Netherlands) *page* 74

Further evaluation of the microhaemagglutination test to determine treponemal antibodies in CSF
S KASATIYA AND A BIRRY (Canada) *page* 77

Assessment of the Till-U-Test GC slide
W H DONALD (Gt Britain) *page* 81

Delay and inhibition of growth of *Neisseria gonorrhoeae* in vitro
WILLIAM G WESTBROOK III (Philippines) *page* 83

Prolonged use of the Greenland method of treatment of gonorrhoea
A J EVANS, G D MORRISON, AND D J PRICE (Gt Britain) *page* 88

Value and feasibility of screening women attending STD clinics for cervical chlamydial infections
SHIRLEY J RICHMOND, I D PAUL, AND P K TAYLOR (Gt Britain) *page* 92

Colonisation of pregnant and puerperal women and neonates with *Chlamydia trachomatis*
P-A MÅRDH, I HELIN, S BOBECK, J LAURIN, AND T NILSSON (Sweden) *page* 96

Treatment of nongonococcal urethritis with trimethoprim sulphadiazine and with placebo
J PAAVONEN, M KOUSA, P A SAIKKU, E VARTIAINEN, L KANERVA, AND A LASSUS (Finland) *page* 101

Carcinoma of the bladder presenting as gonococcal cystitis
A L BLACKWELL AND J BARROW (Gt Britain) *page* 105

Vaginal pH and microflora related to yeast infections and treatment
S M DRAKE, B A EVANS, AND A GERKEN (Gt Britain) *page* 107

Proctitis and herpes simplex virus in homosexual men
D GOLDMEIER (Gt Britain) *page* 111

First African Regional Conference on Sexually Transmitted Diseases
R D CATTERALL (Gt Britain) *page* 115

Correspondence *page* 117

Book review *page* 118

Abstracts *page* 119

ASTM CODEN: BJVDAK 56 (2) 65-124 (1980)
ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

June 1980 Vol. 56 No. 3

Acc
28 10 80

British Journal of
Venereal Diseases

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

R N THIN *(Editor)*
J D ORIEL
B EVANS
A McMILLAN *(Abstracts)*
(Assistant Editors)
C B S SCHOFIELD
(President, MSSVD)

J BARROW
(Hon. Sec., MSSVD)
J WALLIN
G CAUSSE
J M KNOX
A E WILKINSON

I PHILLIPS
G CSONKA
E STOLZ
J THIVOLET
L M DRUSIN

EDITOR
British Medical Journal
SHUNA JUDGE
(Technical Editor)

This *Journal,* founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A E Wilkinson, 22 Kingsend, Ruislip, Middlesex HA4 7DA. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals,* available from the Publishing Manager, *British Medical Journal,* BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; **55:** 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS. *Venereal Diseases.* 3rd ed. London: Baillière and Tindall, 1975.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 214-22.

PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £23.00 in the United Kingdom and Republic of Ireland and US$55.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US$55.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal,* 1172 Commonwealth Avenue, Boston, Mass. 02134. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases,* BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1980 by the *British Journal of Venereal Diseases.* This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.

"Once the infection has been established in the female, it can undoubtedly be transmitted to the male consort... This may, in turn... serve as a reservoir for the reinfection of his partner after her condition had responded successfully to treatment. The risk of this sexual 'ping-pong' has been recognised..."[1]

ECOSTATIN*
Pessaries: Each contain 150 mg econazole nitrate.
Twin Pack: Three pessaries plus 15 g Ecostatin cream. 1% w/w econazole nitrate.

Indications
Pessaries and Twin Pack: Vulvovaginal candidosis.

Actions
Econazole nitrate is a broad-spectrum antifungal agent.

Dosage and Administration
Pessaries and Twin Pack: One pessary to be inserted at bedtime for three consecutive nights. The cream to be applied twice daily to the anogenital area.

Precautions
The pessaries should not be used during the first trimester of pregnancy unless deemed essential for the patient's wellbeing.
Topical preparations should not be used in or near the eyes and used with care in the presence of eczematous dermatitis.

Side-effects
Occasional local irritation. The pessaries may cause transitory discomfort.

Package quantities, prices and Product Licence Numbers
Pessaries: Pack of 3 with applicator – £3.60
PL No. 0085/0013
Twin Pack: 3 pessaries, applicator and 15 g cream – £4.70
—
Cream: 15 g – £1.25; 30 g – £2.50
PL No. 0085/0014

Product Licence Holder
F.A.I.R. Laboratories Ltd.,
179 Heath Road,
Twickenham, TW1 4BJ

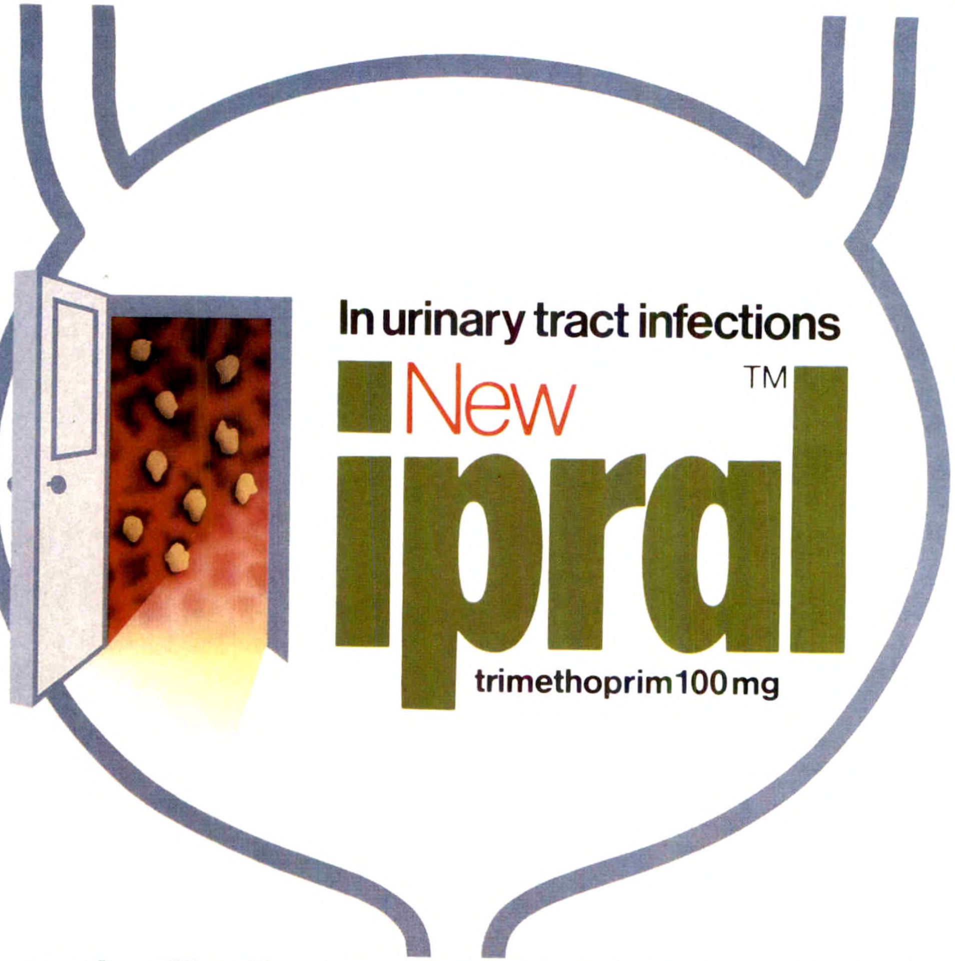
Just one pessary a night for three nights clears candidal vaginitis. The cream treats the anogenital region, and its simultaneous use by the male partner will prevent re-infection.

- **Total pessary disintegration independent of vaginal secretion.**
- **Ecostatin pessaries melt and disperse within ten minutes extensively coating the vaginal wall.**
- **Short course of treatment and acceptable presentation leads to improved patient compliance.**
- **Primary resistance has not been observed.**
- **Twin pack helps prevent re-infection and can be used to treat the sexual partner.**

Further information is available from
FAIR Laboratories Ltd., 179 Heath Road, Twickenham, Middx. TW1 4BJ

**Ecostatin is a registered trademark and FAIR Laboratories is the authorised user.*

[1] **Br. J. Sex. Med. 1974, 1, 21.**



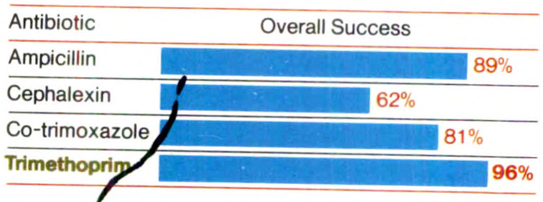
...the way to effective treatment of urinary tract infections

Trimethoprim – proved effective as a single entity without the side-effects associated with sulphonamides.

"Trimethoprim alone may well prove as effective as trimethoprim-sulphamethoxazole combinations ..."[1]

96% success in general practice.[2] Half as many side-effects.

Results of 107 Courses of Treatment in 96 Domiciliary Patients



ipral simple treatment for acute infections – 2 tablets b.d.

ipral for routine use in chronic U.T.I. – 1 tablet b.d.

In a study on recurrent U.T.I.'s
"... trimethoprim is the most effective of the antibacterial agents examined."[3]

Greater efficacy with fewer side effects.

In the trial in acute U.T.I.'s

"... the total side effects caused by ampicillin, cephalexin and co-trimoxazole were similar and occurred twice as often as when trimethoprim was given alone ..."[2]

Antibiotic	No. of Patients	% Side Effects
Ampicillin	88	22
Cephalexin	84	25
Co-trimoxazole	83	21
Trimethoprim	**84**	**8**
Total	339	

Prescribing information

IPRAL tablets

Each tablet contains trimethoprim B.P. 100 mg.

Indications

Ipral is indicated for the treatment and prophylaxis of acute and chronic urinary tract infections.

Actions

Ipral is active in-vitro against most pathogenic Gram-positive and Gram-negative bacteria. Exceptions include Neisseria species and Pseudomonas aeruginosa.

Dosage and administration

For acute urinary tract infections 2 tablets twice daily is recommended. For long term and prophylactic therapy 1 tablet twice daily is recommended.

Contra-indications

Ipral should not be given to patients with severe renal insufficiency where blood levels cannot be monitored regularly. Ipral should not be used in pregnancy or during the first few weeks of life.

Precautions

Care is necessary in administration to patients with impaired renal function. Special precaution should be exercised in patients with a predisposition to folate deficiency.

Side-effects

Nausea, vomiting and skin rashes may occur but are rare. Ipral may depress haematopoiesis during prolonged therapy. This has proved reversible when treated with calcium folinate.

Treatment of overdosage

Gastric lavage. Calcium folinate may be used to counteract any effect of Ipral on bone-marrow.

Package quantities and price

Packs of 100 tablets £5.30.

Product Licence No. - 0034/0190.

Full prescribing information available from:
Technical Services Department, E. R. Squibb & Sons Ltd.
Regal House, Twickenham, TW1 3QT
Tel: 01-892 0164

5280 Oct 79

Ipral is a Trade Mark of E. R. Squibb & Sons Ltd.

...the way to effective treatment of urinary tract infections

- As effective as trimethoprim/sulphamethoxazole combination
- Avoids the side effects of sulphonamides
- At a sensible price

and "Trimethoprim given alone was shown to be much better tolerated."[2]

"Treatment with TMP (trimethoprim) alone is superior to the combined regimen due to a smaller incidence of side-effects; ..." thus "... introduction of TMP alone on the market is recommended."[4]

"Less gastrointestinal side-effects and skin rashes have occurred with trimethoprim ..."[3]

References
1. Lancet 1979, **1**, 493. 2. Br. med. J. 1972, **2**, 673.
3. Ann. clin. Res. 1978, **10**, (suppl. 22). 4. Chemother. 1973, **19**, 314.

New ipral™

trimethoprim 100 mg

more than half the story

SQUIBB™ Full prescribing information available from Technical Services Department, E. R. Squibb & Sons Ltd. Regal House, Twickenham, TW1 3QT. Tel. 01-892 0164

MINOCIN

Minocycline
Hydrochloride
100 mg

TABLETS

for the treatment
of gonorrhoea and
non-specific urethritis[1-4]

---

1 Brit. J. Vener. Dis. (1975), 51, 97–98
2 Brit. J. Vener. Dis. (1975), 51, 206–209
3 Brit. J. Vener. Dis. (1976), 52, 43–45
4 Brit. J. Vener. Dis. (1978), 54, 107–111

INFORMATION ON THE USE OF MINOCIN
IN THE TREATMENT OF VENEREAL DISEASE
AND PRODUCT DATA WILL BE SUPPLIED
UPON REQUEST

Lederle Lederle Laboratories
Research for British Medicine
A division of Cyanamid of Great Britain Ltd
Fareham Road, Gosport, Hants PO13 0AS

MINOCIN is a trademark for minocycline hydrochloride

Br J Vener Dis 1980;**56**:125-8.

Influence of testicular fluid infected with Treponema pallidum on intradermal lesions

T J FITZGERALD AND R C JOHNSON

From the Department of Medical Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota, USA

SUMMARY A viscous mucoid fluid occasionally accumulates after intratesticular inoculation of rabbits with *Treponema pallidum*. Experiments were performed to assess the effects of this testicular fluid on the development of syphilitic lesions. Intramuscular injections of this fluid altered host defences as indicated by shorter incubation periods, by reactivation of healing lesions, and by the presence of lesions at a time when solid immunity should have developed.

Introduction

Mucoid material characteristically accumulates within syphilitic lesions. This material contains acidic mucopolysaccharides as indicated by metachromasia.[1-4] These mucopolysaccharides have been tentatively identified as hyaluronic acid and chondroitin sulphate based on their partial degradation by hyaluronidase.[1 3 4] *Treponema pallidum* (Nichols strain) has an outer layer of amorphous material composed of mucopolysaccharide as shown by reaction with ruthenium red, toluidine blue, acidified bovine serum albumin, wheat-germ agglutinin, and soybean agglutinin.[5-7] Fitzgerald and Johnson[6] suggested that the mucoid material within lesions results from treponemal biosynthesis of this outer mucopolysaccharide layer.

The effects of the mucoid testicular fluid on syphilitic lesions in rabbits were examined with an unexpected result.

Material and methods

The Nichols strain of *T pallidum* was maintained by intratesticular passage in Dutch Belt rabbits. Each testis was inoculated with 10^7 to 3×10^7 treponemes. Within 9-14 days the animals were killed and the organisms were harvested as previously described.[8]

After intratesticular inoculation, a mucoid fluid accumulated within the membranes of some of the infected testes. This testicular fluid (TF) was withdrawn by needle aspiration and 15 mmol/l dithiothreitol was added. The mucoid TF contained numerous actively motile treponemes. Heating of this preparation for 5-7 minutes at 55°C inactivated the motility of the treponemes. This heated preparation was injected intradermally into rabbits to assess virulence. After 60 days no lesions were apparent, indicating that the heating process effectively killed the organisms.

The quantity of acidic mucopolysaccharide within the TF was determined by reaction with acidified bovine serum albumin.[9] Each preparation of TF contained 5-10 mg mucopolysaccharide/ml. One millilitre of heated TF was mixed vigorously with 1 ml incomplete Freund's adjuvant (Difco). This suspension was then injected intramuscularly; each injection contained a total of 5-10 mg mucopolysaccharide. All injections of TF were given intramuscularly in the lower portion of the back legs of the animals. All challenges with freshly harvested preparations of *T pallidum* were injected intradermally on top of the backs of the animals. In separate experiments, the injection of incomplete Freund's adjuvant or the injection of extracts of uninfected rabbit testes did not affect the development of syphilitic dermal lesions.

Results

The effects of TF on syphilitic lesions in three related experiments are summarised in the table.

FIRST EXPERIMENT

In the first experiment, a rabbit was injected intramuscularly with TF containing 5-10 mg mucopolysaccharide. Four weeks later there was no evidence of treponemal lesions at the site of intramuscular injection or at any other site. At this

Address for reprints: Dr T J Fitzgerald, Department of Medical Microbiology, University of Minnesota, 2205 East Fifth Street, Duluth, Minnesota 55812, USA

Received for publication 2 October 1978

TABLE *Decreased incubation periods after intramuscular injection of infected testicular fluid (TF)*

Experiment	*Treponemes/site*	*Animal*	*No positive*/ sites injected*	*Incubation period† (days)*	
				Range	*Mean*
1	10^4	Control	9/9	9-11	10·4
		TF	9/9	3-4	3·7
2	10^3	Control	4/4	13-15	13·5
		TF	4/4	4-6	5·5
3	10^6	Control	8/8	3-4	3·4
		TF	0/8	‡	
		TF	0/8	‡	

*Development of treponemal skin lesions
†Initial detection of erythema and induration
‡No treponemal skin lesions apparent after 60 days

time, this animal and a control animal not injected with TF were inoculated intradermally at nine sites on the shaved back with approximately 10^4 treponemes/site. The incubation period before the appearance of erythema and induration was 9-11 days for the nine sites on the control animal. In the test animal, the incubation period was considerably shortened; all nine sites showed erythema and induration on days 3 and 4. In our laboratory an incubation period of this duration usually occurs after inoculation of approximately 10^6 treponemes/site. In this experiment 10^4 treponemes/site were inoculated.

SECOND EXPERIMENT

First inoculation

In the second experiment, one rabbit was injected intramuscularly with TF containing 5-10 mg mucopolysaccharide. Four weeks later, this animal and a control animal were challenged intradermally at four sites with approximately 10^3 treponemes/ml. As in the previous experiment, a shortening of the incubation period was observed in the TF-injected rabbit. In the control animal the mean incubation period was 13·5 days; in the test animal, it was 5·5 days. An incubation period of 5-6 days usually occurs after inoculation of approximately 10^5 treponemes/site; in this experiment 10^3 treponemes/site were inoculated

Aspirates of lesion material from each site were examined for the presence of actively motile treponemes. The four control and the four test sites gave positive results, containing 1-5 organisms per microscopic field at ×430 magnification.

Development of lesions

As shown in the figure, the development of lesions in each animal progressed in typical fashion and within the expected time periods. Lesions were initially erythematous and slightly indurated; they increased in size and ulcerated within about 10 days. After two weeks the size and severity of ulceration were maximal. The lesions then began to heal. The only observable difference was the shorter incubation period in the TF-injected animal.

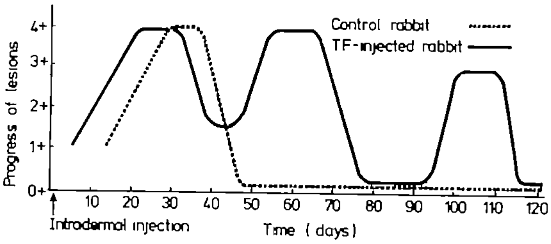


FIGURE *Development of lesions in two rabbits inoculated intradermally on day 0 with 1×10³ treponemes per site. Preparations of heated testicular fluid (TF) mixed with incomplete Freund's adjuvant were injected intramuscularly into one of the animals 30 days before day 0 and on day 36 and day 84. Development of lesions was rated as: 0+, no symptoms; 1+, initial erythema plus induration; 2+, increased size plus erythema and induration; 3+, beginning of ulceration; 4+, ulceration; and 0+, healed.*

Second inoculation

A second preparation of TF containing 5-10 mg mucopolysaccharide was injected intramuscularly into the test animal on day 36. All four dermal lesions continued to heal for approximately eight days. At this point, the healing process was interrupted. The four healing lesions began to increase in size and induration (figure). Darkfield samples of lesion material were examined on day 50. Each of the four samples contained relatively large numbers of actively motile treponemes (10-50 organisms per microscopic field at ×430 magnification).

The four lesions continued to increase in size and severity and again ulcerated. Healing was detectable within a few weeks. The development and time sequences of these lesions were typical for dermal lesions induced by *T pallidum*.

Third inoculation

On day 84, approximately 20 days after healing of the reactivated dermal lesions, a third preparation of TF containing 5-10 mg mucopolysaccharide was injected intramuscularly into the test animal. Within 10 days, three of the four previously healed lesions began to increase in size and induration (figure). Darkfield samples removed on day 105 showed sluggishly motile treponemes within three reactivated sites, but in this case far fewer treponemes were detected (one organism per 10 microscopic fields at ×430 magnification).

The reactivation of these lesions was not as dramatic as that observed after the second injection of TF on day 36. The lesions were not as pronounced in size and severity, although they did ulcerate and healing occurred after a shorter interval.

Further attempts to reactivate the four dermal lesions by injecting preparations of TF intramuscularly on day 106 and day 122 were unsuccessful.

THIRD EXPERIMENT

In the third experiment, two immune rabbits were injected intramuscularly with TF containing 5-10 mg mucopolysaccharide. One animal had been injected intratesticularly with 6×10^7 treponemes three months previously and an obvious orchitis had developed; the second animal had been injected intratesticularly with 6×10^7 treponemes one year previously and had subsequently been challenged eight times with approximately 10^8 treponemes per site at eight sites. Lesions developed in the control animal at all sites on days 3 and 4. In contrast, no lesions were detected after 60 days in either immune animal. Thus, injections of TF in these experiments did not interfere with solid immunity after it had developed in syphilitic rabbits.

Discussion

The interruption of the healing process resulting in reactivation of dermal lesions was most unusual. There is only one other report of reactivation of syphilitic lesions and that involved antibiotic treatment.[10] In experimental syphilis, once lesions begin to heal the healing continues. As shown in the figure, the four lesions on the control animal were completely healed by day 45. Occasionally, disseminated syphilis in rabbits is observed. These disseminated lesions, however, occur at sites other than those initially inoculated intradermally. The reactivation of lesions after intramuscular injection of TF indicates that infectious treponemes were still present within the healing (day 36) and healed (day 84) lesions. TF in some way altered the usual host defences that develop in experimental syphilis, as indicated by shorter incubation periods and by reactivation of lesions. In addition, in this animal lesions were present at a time when solid immunity should have developed (day 90). Apparently, the interference with host defences resulted in re-establishment of the infectious process. Darkfield samples of the reactivated lesions contained motile treponemes, showing that these lesions were due to multiplication of *T pallidum*.

A number of reports[11-16] have speculated that immunosuppression occurs at certain stages of syphilitic infection. This was suggested by depletion of lymphoid cells within lymph nodes and spleens and by depression of cell-mediated immune responses. In the preliminary experiments reported in this paper, no attempt was made to determine the specific component within TF that was responsible for interfering with host defence mechanisms. TF is a complex mixture of both treponemal and host products and it contains large amounts of mucopolysaccharides. *T pallidum* (Nichols strain) has an outer coat of mucopolysaccharide.[5-7] Fitzgerald and Johnson[6] suggested that the mucoid material that accumulates during syphilitic infection results from treponemal biosynthesis of its mucopolysaccharide capsule and that the mucopolysaccharide within TF is primarily of treponemal origin. The compromise of host defences after injection of TF may reflect a type of immunosuppression. If this is the case, then the treponemal mucopolysaccharides might be immunosuppressive in syphilis. This interesting possibility may have direct application to the exacerbation of syphilis that results in the secondary stages of the disease.

Subsequent experiments strongly support the possibility that mucopolysaccharide material is immunosuppressive.[17] TF inhibited the concanavalin-A-induced blast transformation of rabbit lymphocytes. Importantly, the inhibitory effect was destroyed by pretreatment of TF with a commercial preparation of hyaluronidase. This enzyme degrades mucopolysaccharides. In addition, syphilitic rabbit serum inhibited blast transformation. This serum contained mucopolysaccharides, presumably of treponemal origin.[18] Pretreatment of this serum also destroyed the serum inhibitory effect on blast transformation.

This investigation was supported by Public Health Service grants AI-08124 and AI-12978 from the National Institutes of Allergy and Infectious Diseases. We wish to acknowledge the valuable technical assistance of Elizabeth Thompson Wolff.

References

1. Scott V, Dammin GJ. Hyaluronidase and experimental syphilis. III Metachromasia in syphilitic orchitis and its relation to hyaluronic acid. *Amer J Syph* 1950;**34**:501-14.

2. Turner TB, Hollander DH. Cortisone in experimental syphilis. *Johns Hopkins Hosp Bull* 1950;**87:**505-9.
3. Scott V, Dammin GJ. Morphologic and histochemical sequences in syphilitic and in tuberculous orchitis in the rabbit. *Amer J Syph* 1954;**38:**189-202.
4. Turner TB, Hollander DH. Studies on the mechanism of action of cortisone in experimental syphilis. *Amer J Syph* 1954;**38:**371-87.
5. Fitzgerald TJ, Cleveland P, Johnson RC, Miller JN, Sykes JA. Scanning electron microscopy of *Treponema pallidum* (Nichols strain) attached to cultured mammalian cells. *J Bacteriol* 1976;**130:**1333-44.
6. Fitzgerald TJ, Johnson RC. Surface mucopolysaccharides of *Treponema pallidum. Infect Immun* 1979;**24:**244-51.
7. Zeigler JA, Jones AM, Jones RH, Kubica KM. Demonstration of extracellular material at the surface of pathogenic *T pallidum* cells. *Br J Veher Dis* 1976;**52:**1-8.
8. Fitzgerald TJ, Johnson RC, Miller JN, Sykes JA. Characterization of the attachment of *Treponema pallidum* (Nichols strain) to cultured mammalian cells and the potential relationship of attachment to pathogenicity. *Infect Immun* 1977;**18:**467-78.
9. Ibrahim AN, Streitfeld MM. The microassay of hyaluronic acid concentration and hyaluronidase activity by capillary turbidity (CT) and capillary turbidity reduction (CTR) tests. *Ann Biochem* 1973;**56:**428-34.
10. Ito K. A model of experimental syphilitic re-induratio. *Euph Cacoph* 1977;**4:**16-40.
11. Metzger M. The role of immunologic responses in protection against syphilis. In: Johnson RC, ed. *The Biology of Parasitic Spirochetes.* New York: Academic Press, 1976;327-37.
12. Metzger M, Podwinska J, Smogor W. Cell-mediated immunity in experimental syphilis in rabbits. *Arch Immunol Ther Exp* 1977;**25:**25-34.
13. Levene GM, Turk JL, Wright DHM, Grimble AGS. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. *Lancet* 1969;ii:246-7.
14. Wright DHM, Grimble AS. Why is the infectious stage of syphilis prolonged? *Br J Vener Dis* 1974;**50:**45-9.
15. Musher DM, Schell RF, Knox JM. The immunology of syphilis. *Int J Derm* 1976;**15:**566-76.
16. Wicher V, Wicher K. In-vitro cell response of *Treponema pallidum*-infected rabbits. *Clin Exp Immunol* 1977;**24:** 480-500.
17. Bey RF, Johnson RC, Fitzgerald, TJ. Suppression of lymphocyte response to concanavalin A by mucopolysaccharide material from *Treponema pallidum*-infected rabbits. *Infect Immun* 1979;**26:**64-9.
18. Fitzgerald TJ, Johnson RC. Mucopolysaccharidase of *Treponema pallidum. Infect Immun* 1979;**24:**261-8.

Br J Vener Dis 1980;56:129-36.

Sulfhydryl oxidation using procedures and experimental conditions commonly used for Treponema pallidum

T J FITZGERALD, R C JOHNSON, AND E T WOLFF

From the Department of Medical Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota, USA

SUMMARY Certain reducing agents containing sulfhydryl groups are important to the in-vitro survival of *Treponema pallidum*. Discrepancies occur, however, concerning the agents and the concentrations that are optimal. To clarify some of this confusion, sulfhydryl oxidation was determined using procedures and experimental conditions commonly used for *T pallidum*. Sulfhydryl oxidation varied according to the type of culture medium, the size of the culture vessels, the volume of the culture medium, and the gaseous environment within the culture vessels, as well as the method of extracting treponemes from infected testicular tissue. Dithiothreitol maintained highly reduced conditions by reducing disulfide groups to sulfhydryl groups. Lastly, the organisms influenced the sulfhydryl concentration by either direct oxidation or specific uptake. The sulfhydryl content was sharply decreased in the presence of viable preparations of *T pallidum* compared with heated preparations or membrane filtrates of viable preparations.

Introduction

The presence of sulfhydryl (SH) agents prolongs the in-vitro survival of *Treponema pallidum*. Brewer[1] first reported the beneficial effects of sodium thioglycollate on treponemes. Cysteine, glutathione, and thioglycollate were important components of Nelson's basal medium.[2] Weber[3] characterised the influences of SH agents on *T pallidum*. Glutathione, cysteine, thioglycollate, mercaptosuccinate, and homocysteine enhanced survival; mercaptoethanol, ethanethiol, and mercaptoethylamine did not affect survival. Metzger and Smogor[4] reported the effects of various oxidation-reduction potentials on the survival of treponemes. Different combinations and concentrations of glutathione, cysteine, and thioglycollate were used. Optimum survival occurred at Eh −230 to −240 mV (pH 7·25). Another SH agent, dithiothreitol, has recently been added to the list of beneficial agents.[5]

In the last five years, there has been a resurgence of interest in attempts to cultivate *T pallidum* in the presence of tissue culture cells. The diverse findings in published reports on the use of SH agents are shown in table I. In nine separate reports a variety of SH agents at different concentrations was used, whereas in two other reports SH agents were not used.

Reducing agents oxidise in solution. Since the in-vivo generation time of *T pallidum* has been estimated to be 30 hours[16] successful multiplication is likely to require prolonged incubation. Thus, it is important to consider the oxidation of SH groups during this incubation period. In the modified Nelson's medium used by Weber,[3] the glutathione and cysteine were 50% oxidised within four days of incubation. If these agents were partially oxidised before addition of the treponemes, the beneficial effects were sharply decreased. Norris and coworkers[17] also reported that oxidised SH agents were far less beneficial, or even detrimental, to treponemal survival.

The purpose of this report is to clarify some of the present confusion regarding SH agents in tissue culture systems. To this end, SH oxidation was determined using procedures and experimental conditions commonly used for *T pallidum*.

Materials and methods

SULFHYDRYL ASSAY

A modified nitroprusside assay[18] was used to determine low molecular weight SH compounds. A test

Address for reprints: Dr T J Fitzgerald, Department of Medical Microbiology, 2205 East Fifth Street, Duluth, Minnesota 55812, USA

Received for publication 25 June 1979

TABLE 1 *Reducing agents and optimum concentrations for incubation of T pallidum with tissue culture cells*

	Concentration (mmol/l)			
Report	*Glutathione*	*Cysteine*	*Sodium thioglycollate*	*Dithiothreitol*
Fieldsteel *et al*[6]	0·50	0·64		0·25
Fitzgerald *et al*[7]				
Fitzgerald *et al*[8]	4·00	1·00		1·00
Fitzgerald *et al*[5]	4·00	1·00		1·00
Hayes *et al*[9]		6·61	6·14	
Jones *et al*[10]				
Kiraly and Horvath[11]	0·63	0·82	9·61	
Rathlev[12]	1·11			
Sandok *et al*[13]	3·91	6·61	6·14	
Sandok *et al*[14]	0·40	0·68	0·64	
Sandok *et al*[15]	1·95			

sample of 0·5 ml added to 2 ml of water was mixed with 1 ml of 13% (u/v) sulfosalicylic acid. After incubation for 10 minutes in a water bath at 37°C the samples were centrifuged for 10 minutes at 1000× g. A volume of 1·5 ml of the supernatant was removed and 1 ml of K_2CO_2·NaCn and 1 ml of sodium nitroprusside were added. After six minutes at 22°C, the percentage transmittance was read at 525 mmol/l using a Zeiss PM Q11 spectrophotometer. This procedure was highly reproducible; similar readings were recorded within triplicate samples and within control preparations in different experiments.

SH AGENTS

Crystalline-reduced glutathione, cysteine, and dithiothreitol were obtained from Sigma. These reagents were stored in a desiccator at 4°C in an atmosphere of 95% nitrogen and 5% carbon dioxide. Fresh solutions were prepared on the day of the experiment.

GASEOUS ENVIRONMENTS

To test various gaseous environments, experiments were performed in 10-litre desiccator jars that were evacuated and flushed five times with the appropriate gas mixture. One mixture contained 95% nitrogen and 5% carbon dioxide; it is referred to as 0% oxygen. Another mixture contained 2·5% oxygen, 92·5% nitrogen, and 5% carbon dioxide; it is referred to as 2·5% oxygen. These two commercially prepared gas mixtures were obtained from and analysed by Ohio Medical Supply (Cleveland, Ohio).

RABBITS

Adult Dutch Belt rabbits weighing approximately 1·81-2·72 kg were used. Animals were screened for *Treponema paraluis cuniculi* infection by physical examination and by serological testing with the rapid plasma reagin circle card test (Hynson, Westcott, and Dunning, Baltimore, Maryland). Rabbits were maintained at 19-20°C and given antibiotic-free food and water in unrestricted quantities.

T PALLIDUM

The Nichols strain of *T pallidum* was passaged intratesticularly. Each testis received 1×10^7 to 3×10^7 treponemes. Daily intramuscular injections of cortisone acetate (Merck Sharp and Dohme, West Point, Pennsylvania) at 6 mg/kg were given three days after inoculation of testes. After a satisfactory orchitis had developed, usually within 9-11 days, the animals were killed by intracardiac injection of sodium pentobarbital. Testes were removed, rinsed in physiological saline, and placed in tissue culture medium. The basal tissue culture medium contained Eagle's minimal essential medium (MEM) with Hank's balanced salts, 2× amino acids, 2× vitamins, and 4 mmol/l $NaHCO_3$. It was buffered with 30 mmol/l N-2-hydroxyethyl-piperazine-N-2-ethane-sulfonic acid. A supplement of 10% (v/v) heat-inactivated (56°C, 30 minutes) fetal bovine serum (FBS) (Reheis Chemical Co, Kankakee, Illinois) was added. This medium is referred to as Eagle's MEM with 10% FBS. The tissue was sliced and extracted for 10-20 minutes at room temperature under aerobic conditions without shaking. The suspension was centrifuged at 1000× g for seven minutes at 22°C to sediment particulate matter, and the supernatant containing the organisms was decanted. For experiments requiring concentrated suspensions of organisms, this supernatant was then centrifuged at 12 000× g for 30 minutes at 4°C. The pelleted treponemes were resuspended in smaller volumes of the supernatant.

SOURCE AND MAINTENANCE OF CULTURED CELLS

Primary cultures of normal rabbit testis were established as previously described.[7] The basal medium for growth of the cultured cells was identical to that used for the extraction of *T pallidum*.

Results

A standard curve was established using different concentrations of glutathione (GSH) and cysteine

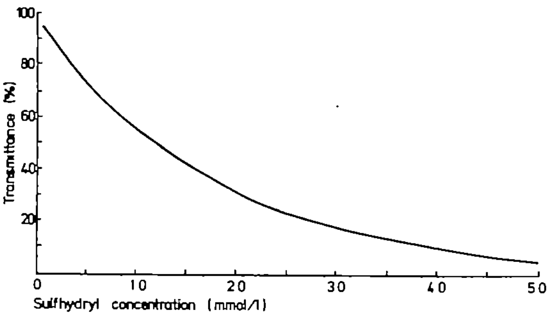


FIGURE *Standard curve for relating percentage transmittance in the nitroprusside reaction to sulfhydryl (SH) concentration.*

(cySH) in saline. Previous studies with tissue culture cells had indicated optimum GSH and cySH concentrations of 4 mmol/l and 1 mmol/l respectively for maintaining survival of *T pallidum*.[5] To establish a standard curve, GSH was tested at 0·33, 0·98, 1·63, 2·61, 3·26, 3·91, and 4·89 mmol/l; cySH was tested at 0·08, 0·83, 1·65, and 2·48 mmol/l. A third preparation contained GSH and cySH at a 4 : 1 ratio at final concentrations of 0·10, 0·37, 1·13, 1·88, 2·62, 3·00, 3·76, 4·88, and 5·00 mmol/l SH. The SH moiety of GSH is cySH. Thus, the nitroprusside assay of the three preparations measures cySH. When the data were plotted on a graph (figure), each of the three curves closely overlapped. To determine SH concentration within test samples, the percentage transmittance was compared to the standard curve.

Standard curves were also established with the SH agents dissolved in basal tissue culture medium instead of saline. Identical data were recorded. Only one difference was apparent. When standing, the SH agents oxidised more quickly in basal medium; oxidation even occurred at −20°C. Thus, in all experiments crystalline GSH and cySH were dissolved in the appropriate medium just before the experiment was begun.

DIFFERENT TREPONEMAL MEDIA

SH oxidation in different treponemal suspending media was examined (table II). GSH (4 mmol/l) and cySH (1 mmol/l) were added to Eagle's MEM, McCoy's medium, 50% normal rabbit serum plus 50% saline, and 50% FBS plus 50% saline. Incubation was performed in air at 36°C in 50-ml screw-cap test tubes containing 20 ml of medium.

TABLE II *Oxidation of SH agents in different treponemal culture media**

	SH concentration (mmol/l) in different media			
Time (h)	*NRS/saline*	*FBS/saline*	*Eagle's MEM*	*McCoy's*
0	5·0	5·0	5·0	4·9
4	2·0	3·4	3·4	3·4
12	1·7	2·5	2·2	3·0
24	0·6	1·6	1·3	2·2
48	0·5	1·0	1·0	1·1
170	0·7	0·9	1·3	1·3

*Air incubation in 50-ml screw-cap test tubes containing 20 ml of medium
NRS = normal rabbit serum
FBS = fetal bovine serum

The most rapid oxidation occurred in rabbit serum (table II). Within four hours, its SH content had decreased to 2 mmol/l compared with 3·4 mmol/l for the other three media. Slowest oxidation occurred in McCoy's medium. After 24 hours, pronounced differences were observed. The SH concentration had decreased to 0·6 mmol/l in rabbit serum, to 1·3 mmol/l in Eagle's MEM, to 1·6 mmol/l in FBS, and to 2·2 mmol/l in McCoy's medium. With continued incubation, SH oxidation slowed considerably. After 170 hours, the SH concentration was 0·7 mmol/l in rabbit serum, 0·9 mmol/l in FBS, and 1·3 mmol/l in both Eagle's MEM and McCoy's medium.

FLUID SURFACE AREA

SH oxidation is related to the amount of fluid surface area in direct contact with the atmosphere. To this end, 15 ml of medium was added to a 600-ml bottle, a T-30 culture flask (75-ml volume), and a 15-ml screw-cap test tube. The medium contained GSH (4 mmol/l) and cySH (1 mmol/l) in Eagle's MEM with 10% FBS. These vessels were incubated in air at 36°C. Owing to the smaller area interface between air and medium, slowest SH oxidation occurred in the test tube (table III). After seven hours, the SH

TABLE III *Oxidation of SH agents in different containers**

	SH concentration (mmol/l)		
Time (h)	*600-ml bottle*	*T-30 flask*	*15-ml test tube*
0	5·0	4·9	5·0
7	1·6	2·4	3·6
19	1·3	1·3	3·2
25	1·3	1·3	2·6
44	1·4	1·4	2·1
66	1·4	1·4	1·6

*Air incubation of vessels containing 15 ml of Eagle's MEM with 10% FBS

TABLE IV *Oxidation of SH agents in different volumes**

	SH concentration (mmol/l)	
Time (h)	*5-ml volume*	*15-ml volume*
0	4·9	4·9
5	3·7	3·8
10	3·2	3·7
20	2·2	3·3
48	0·8	2·5

*Air incubation of 15-ml screw-cap test tubes containing Eagle's MEM with 10% FBS

content within the 600-ml bottle was 1·6 mmol/l compared with 2·4 mmol/l for the T-30 vessel and 3·6 mmol/l for the test tube. After 19 hours, SH concentration in the large bottle and the T-30 flask was 1·3 mmol/l compared with 3·2 mmol/l in the test tube. A plateau of SH oxidation was detected with continued incubation. This plateau was reached within 19 hours in the bottle and T-30 vessel and within 66 hours in the test tube.

DIFFERENT VOLUMES OF MEDIA

SH oxidation should also be related to the volume of medium within the culture vessels. In the next set of experiments, 5 ml and 15 ml of medium were added to 15-ml screw-cap test tubes. The medium contained GSH (4 mmol/l) and cySH (1 mmol/l) in Eagle's MEM with 10% FBS. The tubes were incubated in air at 36°C. Oxidation of SH was much faster in the smaller volume (table IV). After 20 hours, the 5-ml sample contained 2·2 mmol/l SH compared with 3·3 mmol/l in the 15-ml sample. After 48 hours, the 15-ml sample contained three times as much SH as the 5-ml sample (2·5 mmol/l and 0·8 mmol/l respectively).

GASEOUS ENVIRONMENT

In previous tissue culture experiments, treponemes were incubated in different gaseous environments.[5] Experiments were performed to assess SH oxidation within these environments. GSH (4 mmol/l) and cySH (1 mmol/l) in Eagle's MEM with 10% FBS were exposed to air (approximately 21% oxygen), 2·5% oxygen, or 0% oxygen. T-30 culture vessels containing 7 ml of medium were incubated with loose-fitting metal caps at 36°C.

Oxidation of SH was very rapid in air, minimal in 0% oxygen, and intermediate in 2·5% oxygen (table V). After five hours, 2·2 mmol/l SH remained in the medium exposed to air compared with 3·6 mmol/l in 2·5% oxygen and 4·7 mmol/l in 0% oxygen. Pronounced differences were apparent after 20 hours. The sample in air contained 0·9 mmol/l SH compared with 2·6 mmol/l SH in 2·5% oxygen and 4·7 mmol/l SH in 0% oxygen. SH oxidation decreased to its lowest level after 20 hours in air compared with 170 hours in the other two environments.

ADDITION OF DITHIOTHREITOL

Previous studies had indicated that dithiothreitol (DTT) at 1-2 mmol/l prolonged treponemal survival.[5] The beneficial effect of this reducing agent might be related to its ability to reduce disulfide (SS) groups to SH groups.[19] To test this possibility, various concentrations of DTT (0·1-2 mmol/l) were added to Eagle's MEM with 10% FBS containing GSH (4 mmol/l) and cySH (1 mmol/l). Incubation was performed in air in 50-ml screw-cap test tubes containing 15 ml of medium. Far less SH oxidation was observed in the presence of 1 and 2 mmol/l DTT. SH oxidation was marginally decreased at 0·5 mmol/l DTT. No effects on SH oxidation were detected with DTT at 0·1 mmol/l. DTT alone was not reactive in this assay. Apparently, the sulfosalicylic acid precipitated this compound.

SEQUENTIAL ADDITIONS OF DTT

Treponemal survival in the presence of tissue culture cells is enhanced by the addition of 1 mmol/l DTT every 24 hours.[5] Experiments were undertaken to assess SH oxidation in the absence of treponemes. A volume of 15 ml of Eagle's MEM with 10% FBS with

TABLE V *Oxidation of SH agents in different gaseous environments**

	SH concentration (mmol/l)		
Time (h)	*Air*	*2·5% Oxygen*	*0% Oxygen*
0	4·9	5·0	4·9
5	2·2	3·6	4·7
20	0·9	2·6	4·7
48	0·9	1·7	2·7
72	0·9	1·3	2·3
96	0·9	1·0	2·3
170	0·9	0·9	2·0

*Incubation in T-30 culture vessels containing 7 ml of Eagle's MEM with 10% FBS

TABLE VI *Delay of SH oxidation by sequential addition of dithiothreitol (DTT)**

	SH concentration (mmol/l)				
Time (h)	*Flask 1*	*Flask 2*	*Flask 3*	*Flask 4*	*Flask 5*
0	4·9	5·0†	5·0†	5·0†	5·0†
20	3·2	4·7	4·6†	4·7†	4·7†
44	2·0	2·8	4·6	4·6†	4·6†
66	1·6	2·2	2·7	4·5	4·6†
93	1·6	2·2	2·7	4·5	4·6
126	1·2	1·8	1·9	2·5	3·2

*Air incubation in T-30 culture vessels containing 15 ml of Eagle's MEM with 10% FBS and cultured rabbit testis cells
†Added 1 mmol/l DTT

GSH (4 mmol/l) and cySH (1 mmol/l) was incubated in 2·5% oxygen in T-30 culture vessels containing confluent monolayers of normal rabbit testis cells. DTT at 1 mmol/l was added to the various flasks at the times indicated in table VI. Assays for SH groups were performed immediately after the addition of DTT (table VI).

The periodic addition of DTT maintained reduced conditions for extended periods. Fastest SH oxidation occurred in flask 1; slowest SH oxidation occurred in flask 5. The length of incubation required for each flask to decrease to approximately 3·2 mmol/l SH was noteworthy: flask 1, 20 hours; flask 2, 20-44 hours; flask 3, 44-66 hours; flask 4, 93-126 hours; and flask 5, 126 hours.

Two comments about DTT are pertinent. Firstly, the addition of this agent had an immediate effect on SH concentration. For example, samples that contained 1·2 mmol/l SH increased to 2·1 mmol/l SH 30 minutes after addition of DTT. This reduction of SS may have been quicker but the nitroprusside assay required at least 26 minutes to complete. Secondly, the storage of DTT at −20°C did not adversely influence the effectiveness of DTT in reducing SS groups. After storage for two months this agent remained as potent as freshly prepared DTT.

TISSUE EXTRACTION PROCEDURES

Experiments were then performed to investigate SH oxidation using different extraction procedures for harvesting treponemes from testicular tissue. Two infected testes were sliced centrally and equivalent amounts of tissue were placed in different Petri dishes. In one dish, the tissue was carefully sliced with a sharp scalpel. In a second dish, the tissue was cut with scissors and minced with forceps. The tissue in the third dish was cut with scissors and finely ground for 5 minutes with a pestle and mortar. The culture medium contained Eagle's MEM with 10% FBS with GSH (4 mmol/l) and cySH (1 mmol/l). Treponemes were extracted for 30 minutes in air. Each preparation was centrifuged at 1000×g for seven minutes to remove particulate matter. A volume of 5 ml was then placed into T-15 culture vessels without cultured cells and incubated in air. At various intervals, samples were removed and assessed for treponemal motility and for SH content. As a control, culture medium was used that was not exposed to tissue extraction.

The data from the four experiments are presented in table VII. The fastest oxidation was detected in the sample that was finely ground with pestle and mortar. The slowest oxidation, other than the control, was detected in the sliced tissue. After 11 hours, the sliced tissue sample contained 2·2 mmol/l SH compared with 1·1 mmol/l SH in the cut and minced sample and 0·8 mmol/l SH in the finely ground sample. In addition, treponemal motility was apparently affected by the extraction procedure. Best motility occurred in the tissue samples that were sliced; poorest motility occurred in the finely ground samples.

TABLE VII *Oxidation of SH groups as a function of tissue extraction procedures**

	SH concentration (mmol/l)			
Time (h)	*Control*	*Sliced*	*Cut and minced*	*Finely ground*
2	4·0	4·0	4·0	4·0
5	3·9	3·3	2·4	2·4
11	3·3	2·2	1·1	0·8
21	1·5	1·3	0·7	0·5
47	0·8	0·8	0·5	0·2

*Air incubation in T-15 culture vessels containing 5 ml of Eagle's MEM with 10% FBS

OXIDATION/UPTAKE BY T PALLIDUM

To assess the role of *T pallidum* in decreasing the SH content within the culture medium treponemes were extracted from infected tissue in a volume of 55 ml of Eagle's MEM with 10% FBS containing GSH (4 mmol/l) and cySH (1 mmol/l). After extraction and slow-speed centrifugation to remove particulate matter the organisms were pelleted by high-speed centrifugation. The concentrated treponemes were resuspended in 10 ml of the supernatant at approximately 1×10^8 treponemes/ml. This treponemal suspension was equally divided; one-half was heated at 50°C for five minutes to immobilise the organisms. Four T-15 flasks with confluent monolayers of normal rabbit testis cells were used. Viable treponemes were added to one, heated treponemes were added to another, and the supernatant from the high-speed centrifugation that was membrane-filtered (0·2μ pore size) was added to another. The control flask contained culture medium not exposed to testicular extraction. Incubation was performed in 2·5% oxygen. To maintain treponemal survival, DTT was added periodically to each of the four flasks as indicated in table VIII. The data represent the mean of four separate experiments.

The SH content remaining in the flasks containing heated treponemes, high-speed supernatant, and control medium was similar at each time period. The viable treponemal preparation either utilised or was responsible for oxidation of SH groups. This was apparent after incubation for 90 hours. At this time, the viable preparation contained 1·8 mmol/l SH compared with 2·7-3·0 for the other three preparations. After 138 hours, the viable preparation contained only one-third to one-half as much SH as

TABLE VIII *Oxidation or uptake of SH agents by T pallidum or both**

	SH concentration (mmol/l)				*% Motility*
Time (h)	*Control suspending medium*	*Heated treponemes*	*Supernate without treponemes*	*Viable treponemes*	*Viable treponemes*
0	5·0	4·9	4·9	4·9	98
18†	3·7	3·5	3·5	3·5	98
50†	3·2	3·0	3·0	3·0	90
66†	3·7	3·7	3·7	3·5	72
90†	3·0	2·7	2·8	1·8	16
114†	2·9	2·5	2·5	1·3	8
138†	2·0	1·5	1·5	0·7	0

*Incubation in 2·5% oxygen in T-15 culture vessels containing 5 ml of Eagle's MEM with 10% FBS and cultured rabbit testis cells.
†Added 1 mmol/l DTT

the other three preparations (0·7 mmol/l compared with 1·5-2·0 mmol/l). Motile treponemes in the viable preparation were observed for 114 hours.

Discussion

It is well established that certain SH agents are vital to prolong the in-vitro survival of *T pallidum*. As shown in table I, however, differences exist between laboratories concerning the agents and the optimum concentrations. The purpose of this paper was to demonstrate the wide variation in SH content within different experimental systems. Oxidation to SS groups was related to the size of the culture vessels, the volume of fluid in the vessels, the gaseous environment within the vessels, and the treponemal suspending medium. SH oxidation was also dependent on the method of extracting treponemes from infected tissue. These observations emphasise the importance of clearly defining experimental conditions. For example, a specific concentration of glutathione may be beneficial to treponemal survival when incubation is performed in 5-ml test tubes containing 3 ml of medium. This same concentration of glutathione, however, will probably not be optimal if the 3 ml of treponemes are in a larger test tube or a T-30 flask. Furthermore, concentrations of SH agents that are optimal for treponemes in Nelson's medium may not be optimal for treponemes in Eagle's MEM.

Two examples within our laboratory illustrate the problems associated with SH content using different experimental conditions. When treponemes are incubated in a T-30 culture vessel and in a Sykes-Moore chamber, similar periods of survival occur in each vessel (5-7 days). With the T-30 vessel, but not the chamber, it is necessary to add DTT daily. This may reflect faster SH oxidation in the larger vessel. A second example concerns different incubation systems. In a small test tube containing 1 ml of treponemes, better survival occurs in 3% oxygen in the presence of DTT; poorer survival occurs with the combination of DTT, GSH, and cySH.[17] In a T-30 vessel containing cultured cells in 2·5% oxygen this was not observed; best survival occurred with the combination of DTT, GSH, and cySH.

The influences of DTT on SH oxidation were noteworthy. This agent is an important addition to the tissue culture-*T pallidum* system. The initial report of the beneficial effects of DTT indicated that the minimum concentration for prolonging treponemal survival was approximately 1 mmol/l.[5] The data in this report agree with this observation. DTT at 1 mmol/l effectively slowed the oxidation of SH groups. Previous observations had also demonstrated the beneficial influences of daily addition of DTT to the treponeme-cell culture system.[5] This procedure resulted in a significant delay in SH oxidation (table VI).

A major advantage of DTT is its stability. Solutions of glutathione and cysteine rapidly oxidise in air; DTT does not.[19] Furthermore, DTT is oxidised in the process of reducing other SS groups, and oxidised DTT does not further influence the system.[19] The opposite applies to glutathione and cysteine. When these two agents oxidise, they enhance the chemical reaction favouring SS formation.[20-23] This may be one of the reasons why Weber[3] failed to show that sequential additions of cysteine had beneficial effects on treponemal survival.

The precise role of SH agents in prolonging treponemal survival is unknown. Two possibilities have been suggested:[2-4] (a) SH agents poise the redox potential of the suspending medium, partially neutralising oxygen toxicity; and (b) *T pallidum* requires certain SH agents for active metabolic functioning.

In determining optimum concentrations of SH agents, it is important to consider the oxygen-*T pallidum* interaction. Previously, this organism was considered a strict anaerobe. In an important finding, Cox and Barber[24] demonstrated that *T pallidum* (Nichols strain) takes up oxygen, suggesting an aerobic nature. Baseman and co-workers[25] reported maximum retention of treponemal survival in 10-20% oxygen. Fitzgerald *et al*,[5] Fieldsteel *et al*,[6] Norris *et al*,[17] and Sandok *et al*[15] reported maximum treponemal survival in relatively low concentrations of oxygen (less than 5%). SH content, affected by differing experimental conditions, may be the reason for these discrepancies. Two comments are pertinent; firstly, the concentration of dissolved oxygen within the culture medium is related to the concentration of reducing agents. Molecular oxygen reacts with SH groups to yield SS groups and water, thereby decreasing the amount of dissolved oxygen present.[22,23,26] Secondly, the toxicity of oxygen to micro-organisms appears to be mediated through free radicals.[27] SH groups are able to scavenge free radicals.[22,23,28] In the process of interacting with the SH moiety, the free radical is neutralised, resulting in SS formation. Thus, SH agents conceivably minimise oxygen toxicity by inactivating free radicals. Incubation in the presence of increasing concentrations of oxygen would probably require increasing concentrations of SH agents to counteract oxygen toxicity.

In support of this, Norris and co-workers[17] recently examined the survival of *T pallidum* (Nichols strain) in 21%, 3%, and 0% oxygen in the presence and absence of SH agents. Poor survival was observed under aerobic conditions (21% oxygen) with and without SH agents. Interestingly, directly opposing effects were apparent in the presence of two lower oxygen concentrations. Maximum treponemal survival occurred in 0% oxygen without SH agents. In contrast, with dithiothreitol the treponemes survived for much longer periods in 3% oxygen than in 0% oxygen.

Besides control of oxygen toxicity, SH groups may be critical to metabolic functioning of *T pallidum*. Weber[3] examined the effects of different SH agents on treponemal survival. Some were beneficial and some were not. Since the ineffective agents also lower redox potential, he stated that control of redox potential could not be the sole function of these agents. In the present report the data in table VIII indicate the direct interaction of SH agents with *T pallidum*. SH content in viable treponemal preparations was sharply decreased compared with non-viable preparations or with membrane filtrates of viable preparations. Viable *T pallidum* either oxidises or takes up the SH agents, thereby decreasing their concentration in the suspending medium.

Thus, it appears as if successful in-vitro multiplication will require a delicate balance provided by specific concentrations of various SH agents. These concentrations will have to be carefully balanced and maintained during prolonged periods of in-vitro incubation.

Funds for this study were provided by Public Health Service grants AI-08124 and AI-12978 from the National Institutes of Health, Allergy and Infectious Diseases Branch.

References

1. Brewer JH. Clear liquid mediums for "aerobic" cultivation of anaerobes. *J Amer Med Assoc* 1940; **115**: 598-600.
2. Nelson RA. Factors affecting the survival of *Treponema pallidum* in vitro. *Amer J Epidemiol* 1948; **48**: 120-32.
3. Weber MM. Factors influencing the in-vitro survival of *Treponema pallidum*. *Amer J Epidemiol* 1960; **71**: 401-17.
4. Metzger M, Smogor W. Study of the effect of pH and Eh values of the Nelson-Diesendruck medium on the survival of virulent *Treponema pallidum*. *Arch Immunol Ther Exp* 1966; **14**: 445-53.
5. Fitzgerald TJ, Johnson RC, Sykes JA, Miller JN. Interaction of *Treponema pallidum* (Nichols strain) with cultured mammalian cells: effects of oxygen, reducing agents, serum supplements, and different cell types. *Infect Immun* 1977; **15**: 444-52.
6. Fieldsteel HA, Becker FA, Stout JG. Prolonged survival of virulent *Treponema pallidum* (Nichols strain) in cell-free and tissue culture systems. *Infect Immun* 1977; **18**: 173-82.
7. Fitzgerald TJ, Miller JN, Sykes JA. *Treponema pallidum* (Nichols strain) in tissue cultures: cellular attachment, entry, and survival. *Infect Immun* 1975; **11**: 1133-40.
8. Fitzgerald TJ, Johnson RC, Miller JN, Sykes JA. Characterization of the attachment of *Treponema pallidum* (Nichols strain) to cultured mammalian cells and the potential relationship of attachment to pathogenicity. *Infect Immun* 1977; **18**: 467-78.
9. Hayes, NS, Muse KE, Collier AM, Baseman JB. Parasitism by virulent *Treponema pallidum* of host-cell surfaces. *Infect Immun* 1977; **17**: 174-86.
10. Jones RH, Finn MA, Thomas JJ, Folger C. Growth and subculture of pathogenic *T pallidum* (Nichols strain) in BHK-21 cultured tissue cells. *Br J Vener Dis* 1976; **52**: 18-23.
11. Kiraly K, Horvath I. Survival of *T pallidum* under microaerophilic conditions in cell and tissue cultures. *Zentralbl Bakteriol (Orig A)* 1976; **235**: 500-5.
12. Rathlev T. Investigations of in-vitro survival and virulence of *Treponema pallidum* under aerobiosis. *Br J Vener Dis* 1975; **51**: 296-300.
13. Sandok PL, Jenkin HM, Graves SR, Knight ST. Retention of motility of *Treponema pallidum* (Nichols virulent strain) in an anaerobic cell culture system and in a cell-free system. *J Clin Microbiol* 1976; **3**: 72-4.
14. Sandok PL, Knight ST, Jenkin HM. Examination of various cell culture techniques for coincubation of virulent *Treponema pallidum* (Nichols strain) under anaerobic conditions. *J Clin Microbiol* 1976; **4**: 360-71.
15. Sandok PL, Jenkin HM, Matthews HM, Roberts MS. Unsustained multiplication of *Treponema pallidum* (Nichols virulent strain) in vitro in the presence of oxygen. *Infect Immun* 1978; **19**: 421-9.
16. Magnuson HJ, Eagle H, Fleischmann R. The minimal infectious dose of *Spirochaeta pallida* (Nichols strain) and a consideration of its rate of multiplication in vivo. *Amer J Syph* 1948; **32**: 1-18.
17. Norris SJ, Miller JN, Sykes JA, Fitzgerald TJ. Influence of oxygen tension, sulfhydryl compounds, and serum on the motility and virulence of *Treponema pallidum* (Nichols strain) in a cell-free system. *Infect Immun* 1978; **22**: 689-97.
18. Mortensen E. The nitroprusside method for the determination of reduced glutathione. *Scand J Clin Lab Invest* 1964; **1**: 87-97.
19. Cleland WW. Dithiothreitol, a new protective reagent for SH groups. *Biochem* 1964; **3**: 480-2.

20. Eldjarn L. Some biochemical effects of S-containing protective agents and the development of suitable SH/SS systems for the in vitro studies of such effects. *Prog Biochem Pharmacol* 1965; **1**: 173-85.
21. Jellum E. The prevention of thio autoxidation in biological systems by means of thiolated sephadex. *Acta Chem Scand* 1964; **18**: 1887-95.
22. Jocelyn PC. *Biochemistry of the SH Group*. New York: Academic Press, 1972.
23. Trowell OA. Cells and tissues in culture. In: Willmer EM, ed. *Methods, Biology, and Physiology*, vol 3. New York: Academic Press, 1963: 64-149.
24. Cox CD, Barber MK. Oxygen uptake by *Treponema pallidum*. *Infect Immun* 1974; **10**: 123-7.
25. Baseman JB, Nichols JC, Hayes NS. Virulent *Treponema pallidum*: aerobe or anaerobe? *Infect Immun* 1976; **13**: 704-11.
26. Horn RS, Haugaard N. Inhibition of carbohydrate metabolism by oxygen and N-ethylmaleimide in rat heart homogenates. *J Biol Chem* 1966; **241**: 3078-82.
27. Morris JG. The physiology of obligate anaerobiosis. *Adv Microb Physiol* 1975; **12**: 169-246.
28. Guzman-Barron ES. Oxidation of some oxidation-reduction systems by oxygen at high pressures. *Arch Biochem Biophys* 1955; **59**: 502-10.

Br J Vener Dis 1980;56:137-43.

Ultrastructure of vascular and connective tissue changes in primary syphilis

TERESA WRZOLKOWA* AND JERZY KOZAKIEWICZ†
*From the *Electron Microscopy Laboratory and †Dermatology Clinic, University of Gdansk Medical School, Gdansk, Poland*

SUMMARY Electron microscopical studies of primary syphilitic lesions showed intensive changes in the capillaries in the form of endothelial proliferation and extensive infiltration of different cells in the surrounding connective tissue. The degenerative changes in axon terminals could explain the painless course of primary induration.

Introduction

Until recently there have been few published reports on the ultrastructure of the syphilitic chancre, most of them dealing with the behaviour of spirochaetes in the early phases of infection.[1-5]

The aim of this investigation was to define the character of the abnormalities in the capillaries of the skin and in the surrounding connective tissue in primary syphilitic foci.

Patients and methods

Skin specimens from seven patients treated in the dermatology clinic of the Medical Academy of Gdansk between 1974 and 1978 were examined. Three patients had sero-positive primary syphilis and four had sero-negative primary syphilis (negative results to lipoidal antigen tests, although the titre of the fluorescent treponemal antibody absorbed test varied from 1/450 to 1/1300). The primary changes localised on the prepuce or the glans penis had been present for 3-6 days before examination by electron microscopy in three cases and for 16-20 days in the remaining cases.

The specimens were immediately fixed with 6·25% glutaraldehyde in 0·1 mol/l cacodylate buffer (pH 7·4); postfixation was performed in a similarly buffered 2% solution of osmium tetroxide and embedded in Epon 812. Ultrathin sections were stained with lead citrate and uranyl acetate. They were viewed in a JEM 7A electron microscope.

Address for reprints: Dr T Wrzołkowa, Electron Microscopy Laboratory, Gdansk University Medical School, Debinki 1, 80-211 Gdansk, Poland

Received for publication 27 April 1979

Results

ENDOTHELIAL PROLIFERATION

Electron microscopical studies of the syphilitic chancres showed considerable changes in the capillaries, mainly in the form of endothelial proliferation obliterating the vascular lumen. The cytoplasm of most of these cells showed features of stimulation with numerous mitochondria, ribosomes, and contractile fibres present and a proliferation of rough and smooth endoplasmic reticulum. Some of the endothelial cells were only swollen and showed no signs of proliferation. The coexistence of both types of changed endothelial cells in a single capillary section was noticeable.

The basement membrane of the capillaries was often multilaminated and thickened segmentally (figs 1 and 2). No electron-dense deposits were observed.

Between the layers of the basement membrane homogeneous material of moderate electron-optical density, numerous undifferentiated fibres and fibrils, occasional fibrin threads, and deposits of proteoglycans were visible.

CELLULAR INFILTRATION

The proliferation of pericytes was as prominent as that of the endothelial cells. A characteristic feature of the primary syphilitic foci was an extensive infiltration of lymphocytes, plasma cells, histiocytes, and mast cells. In places some of the cells were closely packed, leaving only an occasionally visible intercellular space between the cell membranes (fig 3). Such an interaction of cells was seen between macrophages, lymphocytes, and plasma cells.

Intensive cellular infiltration was usually observed in specimens from older foci. In two cases, the cellular infiltration was scanty. In the perivascular space extensive agglomerations of a homogeneous or

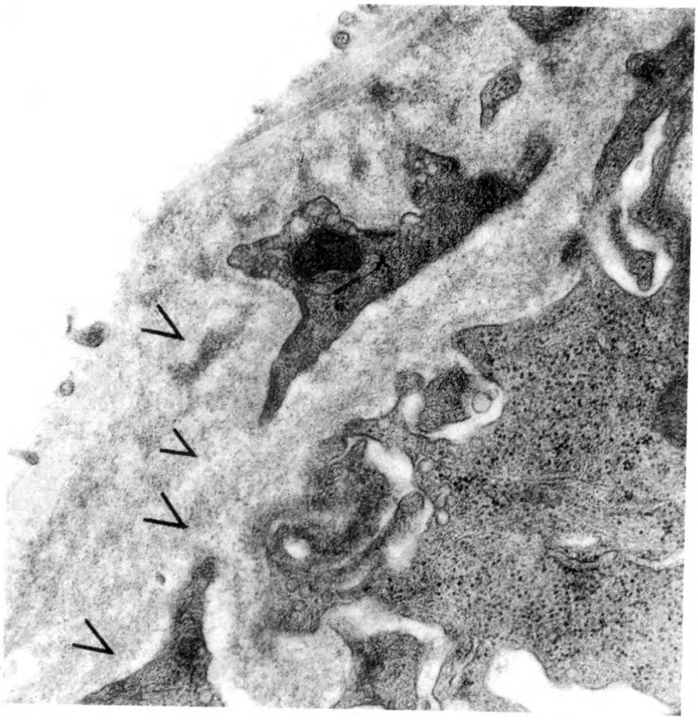


FIG 1 *Fragment of capillary with stimulated endothelial cell and multilayered (>) basement membrane. ×27 000 magnification.*

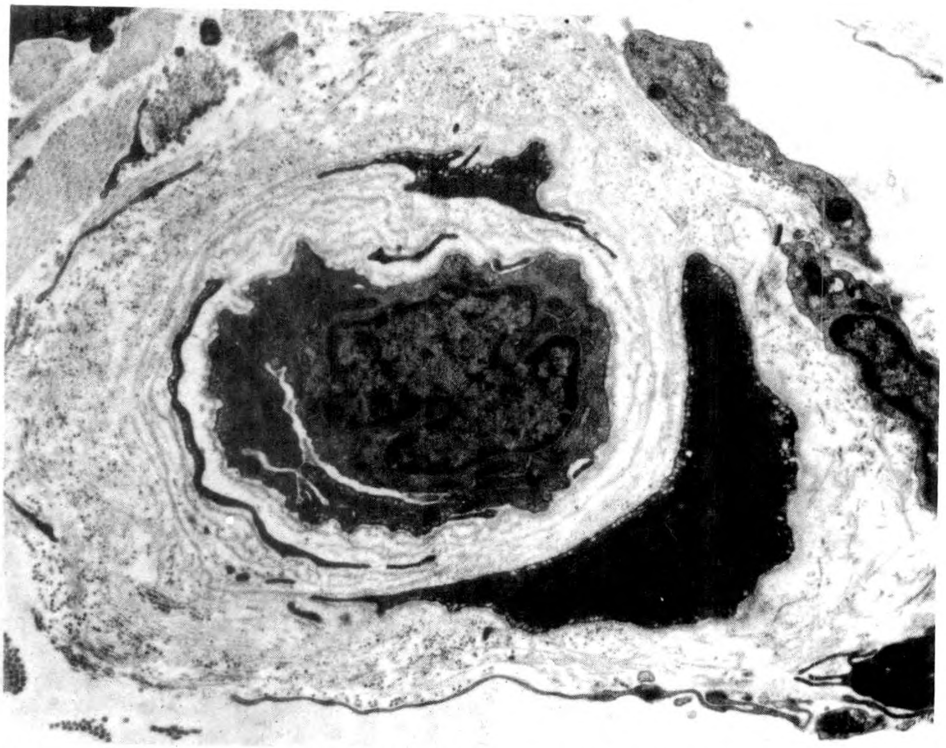


FIG 2 *Capillary vessel with a thick multilayered basement membrane. ×7000 magnification.*

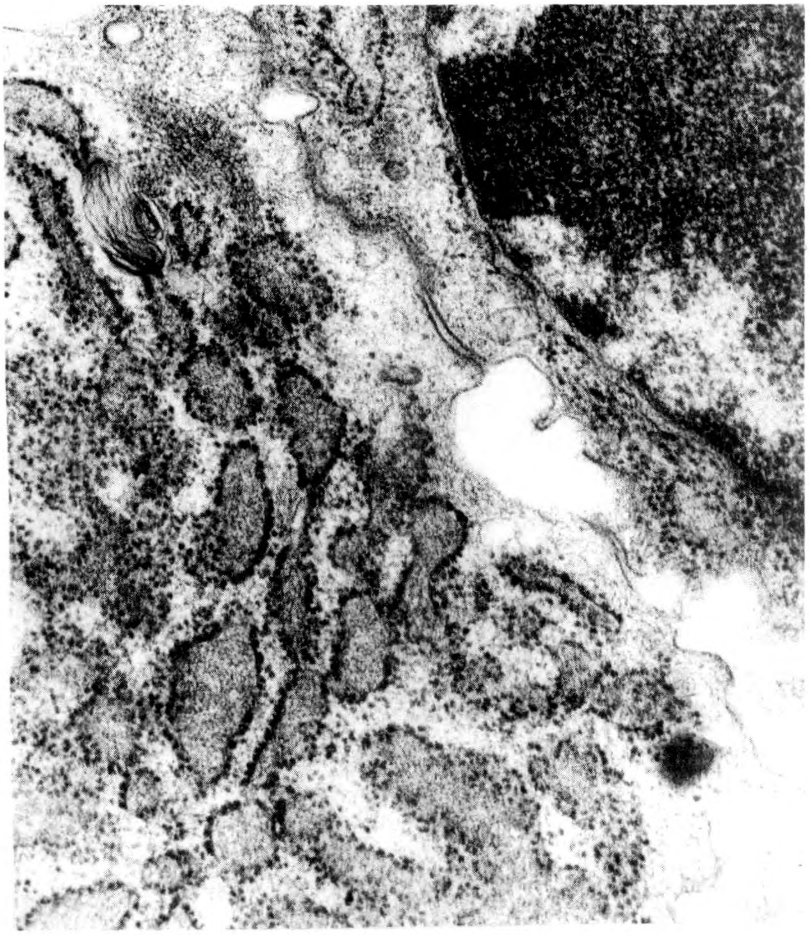


FIG 3 *Closely opposed inflammatory cells with their cell membranes partially invisible. ×46 000 magnification.*

finely granular material were visible. This material was of moderate electron-optical density with numerous thread-like deposits, probably of fibrin.

In most of the studied specimens undifferentiated fibrils and elastic and collagen fibres in different phases of development were present in the perivascular area, usually within the region of infiltration.

Some of the axon terminals showed signs of a degenerative process (fig 4).

TREPONEMES

Treponemes were present in all sections studied. They were most numerous in the capillary walls between the endothelial and pericytic cells.

In the region of infiltration and in the epidermis extracellular treponemes were visible near the cell membranes (fig 5). In areas of close approximation of the treponemes, the cell membrane was indented (fig 6).

Treponemes and fragments of their outer envelope (fig 7) were also present in the exudate outside the area of infiltration. Intracellular treponemes were rarely observed (figs 8 and 9). Fragments of their bodies were surrounded by cytoplasmic processes forming a kind of niche (fig 8). Sometimes they were found in the cytoplasm in vacuoles (fig 9). The intracellular organisms were always devoid of the external envelope, fragments of which were visible in the intercellular spaces (fig 7).

Discussion

The aim of this report was to explain the character of the lesions in the capillaries of the skin in primary syphilitic lesions. The response of the vessels was complex, some of the endothelial and pericytic cells showing proliferation, others being only swollen. Both types of reaction were visible in a single capillary. This could be explained by the variable

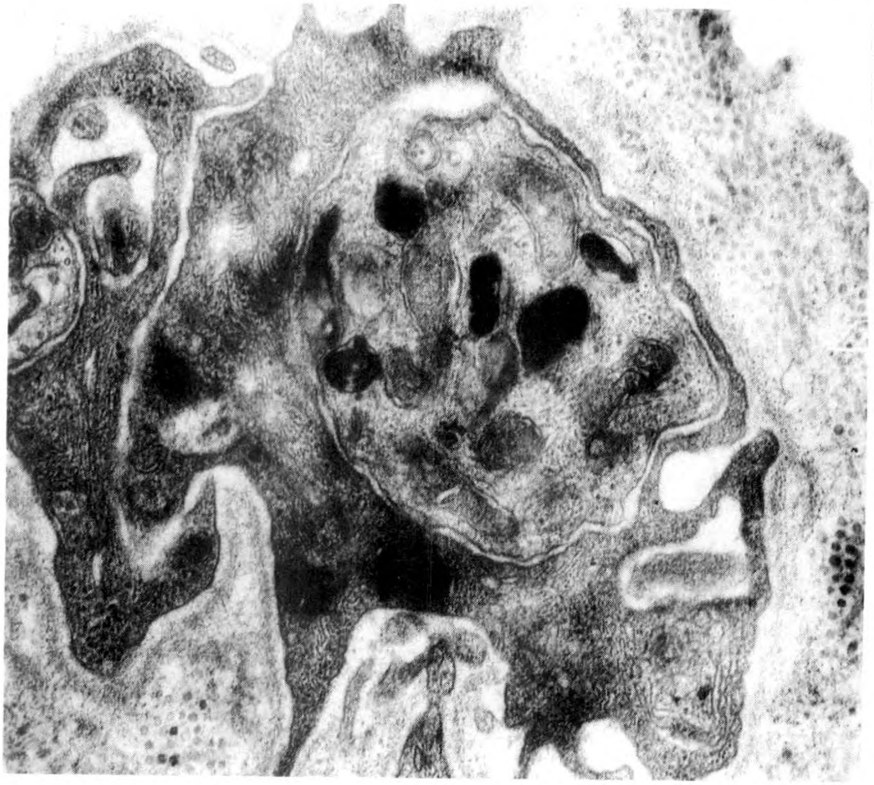


FIG 4 *Fragment of degenerated axon terminal. × 36 000 magnification.*

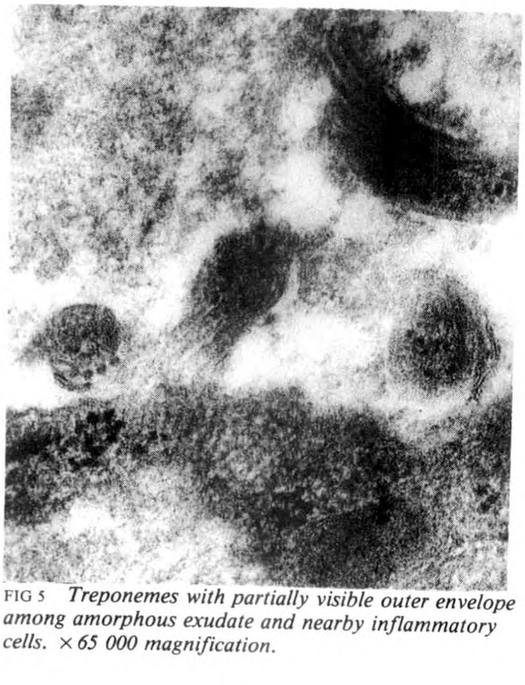


FIG 5 *Treponemes with partially visible outer envelope among amorphous exudate and nearby inflammatory cells. × 65 000 magnification.*

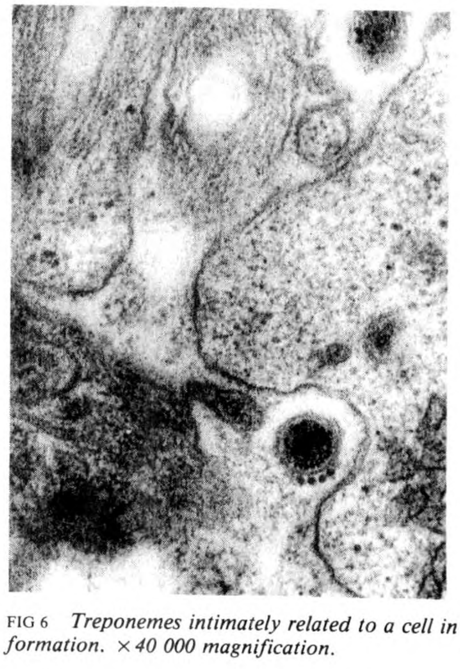


FIG 6 *Treponemes intimately related to a cell in a niche formation. × 40 000 magnification.*

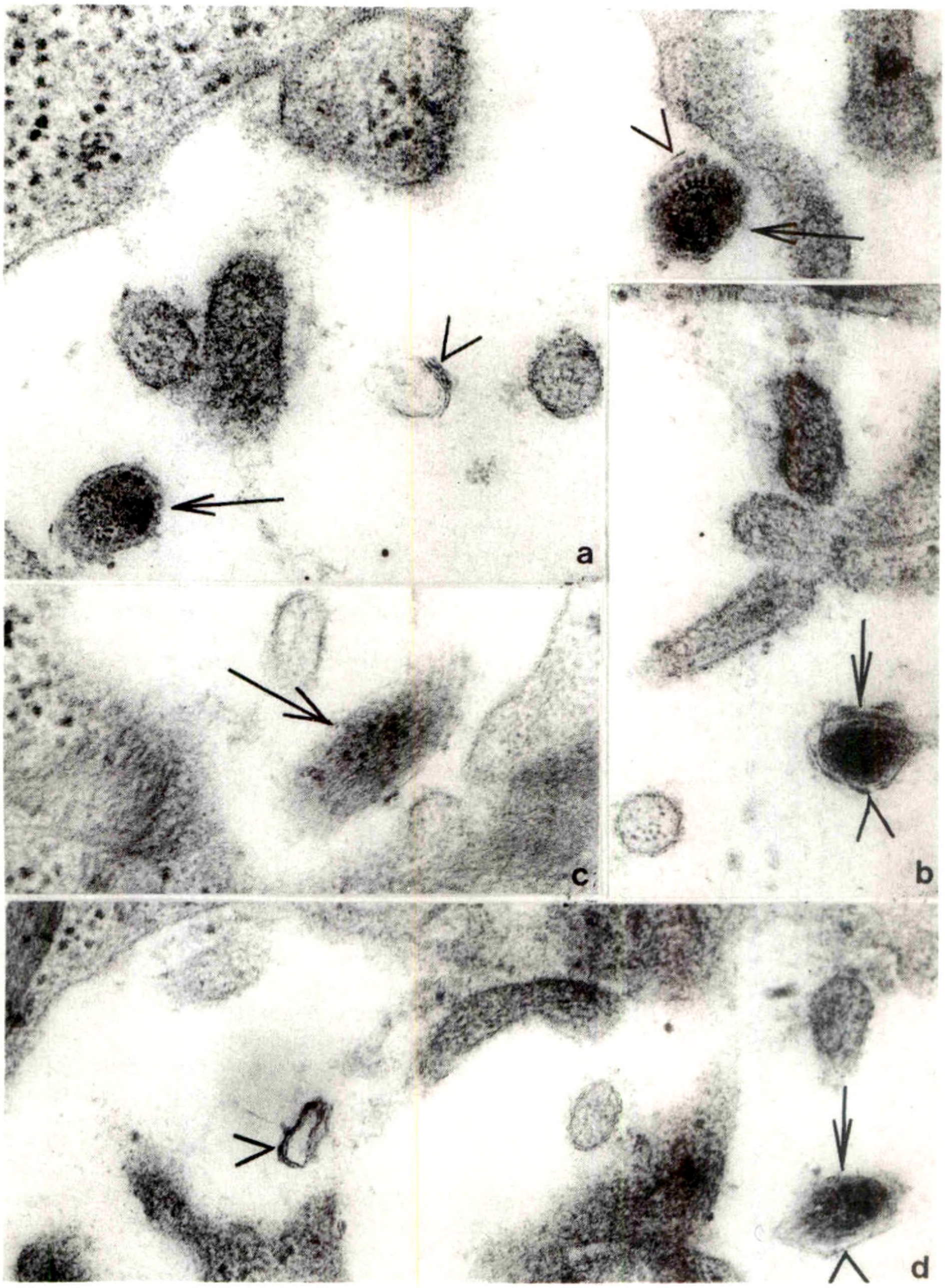


FIG 7 *Treponemes in the extracellular space. (Arrows indicate treponemes.) Outer envelope (>) attached or separated from treponeme. (a) × 75 000; (b) × 67 000; (c) × 75 000; and (d) × 60 000 magnification.*

intensity of the toxic factor or the different functional state of the cells at the moment of injury. It is difficult to exclude other factors influencing the function of the cell.

The presence of treponemes inside the capillaries has been reported by a number of authors.[3 4] The basement membrane of most of the capillaries in the sections was multilaminated. This type of basement membrane is characteristic of the venous capillary. Braverman and Yen[6] described similar basement membranes in the vessels of the papillary and subpapillary layers of the skin in psoriasis.

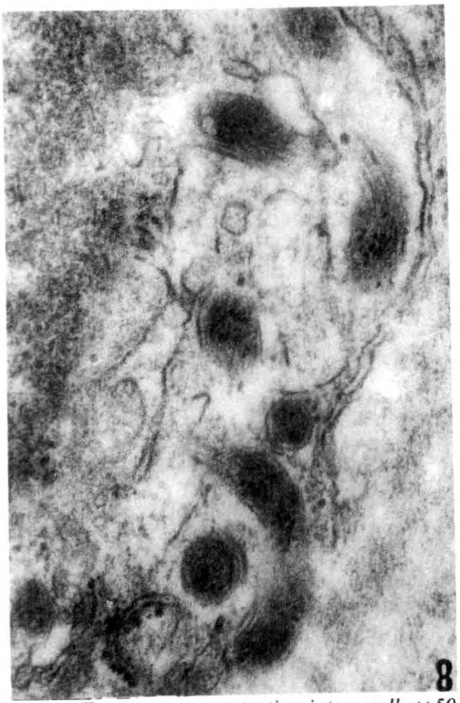


FIG 8 *Treponemes penetrating into a cell. ×50 000 magnification.*

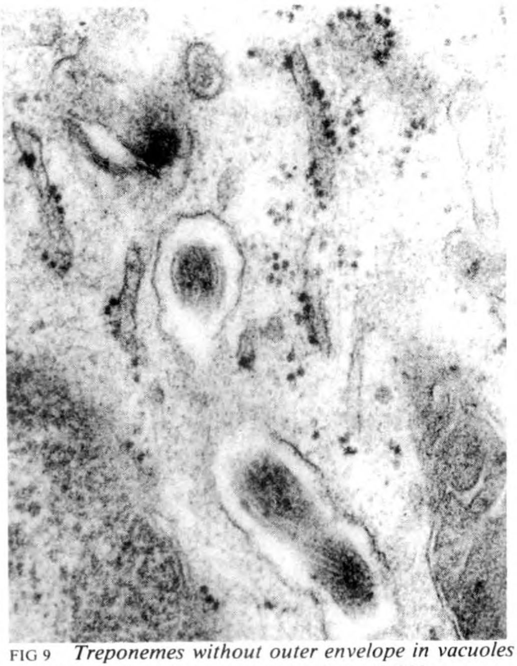


FIG 9 *Treponemes without outer envelope in vacuoles in cytoplasm of an inflammatory cell. ×47 000 magnification.*

In the primary syphilitic focus such a changed basement membrane was visible in the infiltrated area of the dermal papillae and the subpapillary dermis. It is probable that this is a structural reaction of the basement membrane to the inflammatory process.

The cell reaction in the perivascular area varied in intensity. In some of the cases numerous inflammatory cells were present in the primary syphilitic focus. Some of these, especially the macrophages, lymphocytes, and plasma cells, displayed features suggesting interaction, their cell membranes being intimately related in places. According to Twomey and co-workers[7] this phenomenon could be a characteristic of one of the phases of the immunological reaction. In other cases the cellular infiltration was not prominent, the perivascular space being oedematous with numerous fibrin threads. This weak cell response and an extensive exudative inflammatory component, with the presence of thread-like clumps of fibrin, appeared in early stages of primary syphilis when the primary induration had been present for only a few days. Similar changes in older foci could be explained as a state of disturbed balance between the aetiological factor and the defence mechanisms of the human organisms due to the variable number or virulence of the treponemes.

The intensive formation of filaments, collagen, and elastic fibres was characteristic of foci of longer duration.

The degenerative process in the terminal axons seen in our studies has been previously reported in studies on experimental syphilis in rabbits.[8] This process was observed in axon terminals not associated with vessels or muscle cells, probably being sensory axons. This could explain the painless course of the primary induration.

The intercellular and intracellular presence of fragments of treponemes in the area of cellular infiltration and their location in the capillary walls has been observed in previous studies.[2-4]

The present study shows that the outer envelope of the treponemes was often destroyed and separated from the rest of the treponemal body in the intercellular space (fig 7). The outer envelope was never visible in organisms situated intracellularly. Fragments of their bodies were visible in oval and round vacuoles. It has been suggested that treponemes enter the cytoplasm by way of endocytosis.[9] Phagocytosis observed in experimental syphilis seems to support this view. During this process macrophages were seen to surround the organisms with cytoplasmic processes, engulfing them within the cell in vacuoles.[4 5]

Brause and Roberts[10] reported different findings.

They observed in vivo that the spirochaetes concentrated around the macrophages, which showed no phagocytic reaction. At the same time the macrophages quickly absorbed molecules of latex used in the experiment. The authors believed that live mobile treponemes possess antiphagocytic surface factors which prevent their phagocytosis. If these factors were connected with the outer envelope of the organism, the destruction or separation of this envelope—as observed in our studies—could cause endocytosis of the treponemes by appropriate cells. As an effect of this process they become visible intracellularly in oval areas surrounded by an indentation of the cell membrane. In view of the studies carried out by Brause and Roberts,[10] and of our own reports, it seems that the weakening of the biological properties of the treponemes and the damaging of their structure are essential for their phagocytosis by appropriate cells.

References

1. Azar H, Pham T, Kurban A. An electron microscopic study of a syphilitic chancre. *Arch Pathol* 1970;**90:**143-50.
2. Hasagawa T. Electron microscopic observations on the lesion of condyloma latum. *Br J Dermatol* 1969;**81:**367-74.
3. Kozakiewicz J, Wrzołkowa T. *Treponema pallidum* in lesion of early syphilis. *Annals Med Sect Pol Acad Sci* 1976;**21:**63-4.
4. Metz J, Metz G. Elektronenmikroskopischer nachweis von *Treponema pallidum* hautefflorescezen der unbehandelten lues I und II. *Arch Dermatol Forsch* 1972;**243:**241-54.
5. Sykes J, Miller J, Kalan A. *Treponema pallidum* within cells of primary chancre from a human female. *Br J Vener Dis* 1974;**50:**40-4.
6. Braverman L, Yen A. Ultrastructure of the human dermal microcirculation. *J Invest Dermatol* 1977;**68:**44-52.
7. Twomey JJ, Sharkey O Jr, Brown JA, *et al.* Cellular requirements for the mitotic response in allogeneic mixed leucocyte cultures. *J Immunol* 1970;**104:**845-53.
8. Owczynnikow N, Delektorskij V. *Treponema pallidum* in nerve fibres. *Br J Vener Dis* 1975;**51:**10-4.
9. Musher DM, Izzat NN, Mink KW, *et al.* In vitro phagocytosis of avirulent *T pallidum* by rabbit macrophages. *Acta Dermato Venereol* 1972;**52:**349-52.
10. Brause B, Roberts R. Attachment of virulent *Treponema pallidum* to human mononuclear phagocytes. *Br J Vener Dis* 1978;**54:**218-24.

Br J Vener Dis 1980;56:144-7.

Identification of Neisseria gonorrhoeae in the routine venereological laboratory

Comparative study of coagglutination, direct immunofluorescence, and sugar fermentation reaction

T RUFLI

From the University Skin Clinic, Kantonsspital, Basel, Switzerland

SUMMARY The coagglutination (CoA) method for the identification of *Neisseria gonorrhoeae* colonies grown on selective culture media was used on 116 strains in a routine venereological laboratory together with the direct immunofluorescence (IF) test and the sugar fermentation reaction. Correlation of results between the CoA method and the direct IF test and between the CoA test and the sugar fermentation reaction was 95·7% and 97% respectively. The sugar fermentation reaction requires subcultures and is more time-consuming than the IF test; the latter needs elaborate technical equipment and experience. The CoA method however can be carried out with the primary culture, is technically easy to perform and to reproduce, and the result is available within minutes.

Introduction

Culture of *Neisseria gonorrhoeae* has become much simpler since the introduction of the selective media by Thayer and Martin.[1] These media may be obtained commercially at reasonable prices and have proved efficient. The characteristic morphological criteria for the colonies grown on the selective media, in combination with Gram staining and oxidase reaction, allow a preliminary classification of the organisms as *Neisseria*. However, neisseriae other than *N gonorrhoeae* can occur in pharyngeal and rectal smears, for example *Neisseria meningitidis* and *Neisseria lactamica,* both of which are found with increasing frequency in urethral and cervical smears.[2-6] For this reason, a clear classification of neisseriae is needed in venereological investigations.

Dunn and Gordon[7] observed in 1905 the varying ability of the different species of *Neisseria* to degrade sugars. Since 1906 this sugar fermentation test has been used with indicator and sugar-containing media as the method of choice for the identification of the different neisserial strains.[8] The CTA medium[9] is frequently used today with sugar-impregnated filter discs serving as fermentation substrate. The fermentation reaction in a sugar solution without renewed subcultivation (non-growth carbohydrate degradation) is technically demanding and requires highly purified sugar solutions. For these reasons this method is not used in the routine laboratory.[10]

The immunological identification of *N gonorrhoeae,* introduced by Deacon and co-workers,[11] has been used successfully.[12-20] The commercially available FITC-labelled antiserum produced by Difco Laboratories was used in these studies.

Danielsson and Kronvall[21] adapted the method of coagglutination (CoA), originally introduced by Kronvall[22] for streptococci, to *N gonorrhoeae* and found highly specific results with this organism. This method is based on the ability of certain strains of staphylococci to bind immunoglobulins non-specifically to their surface. Addition of the corresponding antigen causes a visible agglutination. Menck[23] successfully evaluated the now commercially available product (Phadebact® Gonococcus Test, Pharmacia AB) using laboratory strains of *N gonorrhoeae* and other *Neisseria* as well as many clinically isolated strains. Barnham and Glynn[24] also evaluated the Phadebact reagent for gonococci on a larger series of gonococci and other neisseriae.

In the present investigation, the identification of 116 consecutive strains of Gram-negative, oxidase-positive diplococci was carried out simultaneously by the direct immunofluorescence (IF) test, by the sugar

Address for reprints: Dr T Rufli, University Skin Clinic, Kantonsspital, CH 4031 Basel, Switzerland

Received for publication 21 August 1979

fermentation reaction, using the method of Stacey and co-workers,[25] and by the CoA method. The latter method aims merely at a rapid and easy identification of *N gonorrhoeae.*

Patients and methods

During the period, 1 April to 15 June 1978, 116 strains of *Neisseria* (61 isolated from 57 male and 55 from 37 female patients at this clinic) were studied. Specimens taken from the patients were immediately inoculated on to Thayer-Martin selective medium[1] (modified by Martin *et al*[26] and produced by Orion Laboratories) and incubated at 36·5°C in a candle jar. The characteristic morphology of the colonies and the positive results of the oxidase reaction and Gram-staining (Gram-negative diplococci) were used as criteria for further investigation.

SUGAR DEGRADATION

The ability of the organisms to degrade sugar was tested on a CTA medium (cystein-tryptic-agar 28·5 g/l; GC agar Difco 10 g/l; NaOH 1 m 10 drops and phenol red as indicator; reagents, Difco) using the method of Thayer.[9] Filter discs impregnated with glucose, maltose, and sucrose were placed on the agar. Sugar degradation was shown by the change of the indicator around the discs to yellow.

IF TEST

The IF test was carried out with FITC-conjugated anti-*N gonorrhoeae* serum (Difco). A characteristic colony of the primary culture was suspended in physiological saline and spread on a slide, allowed to dry, and then fixed by heat. Incubation with the conjugate diluted 1/4 was undertaken at 37°C for 30 minutes. After being washed with PBS and distilled water, the preparation was mounted with Bacto (Difco) mounting-fluid and examined.[16]

COAGGLUTINATION TEST

The CoA test was performed with the Phadebact ® Gonococcus Test (Pharmacia AB). A few colonies of *Neisseria* from the primary culture were suspended in a drop of the test solution containing killed staphylococci coated with anti-*N gonorrhoeae* IgG on a glass slide. A reagent containing staphylococci coated with non-specific IgG served as control. The suspensions were carefully mixed. The result may be read within a minute, a positive reaction consisting of a distinct coagglutination.

TABLE I *Sites of isolation of the 116 strains of Neisseria gonorrhoeae*

	Site of isolation				
Patients	*Urethra*	*Cervix*	*Rectum*	*Tonsils*	*Total*
Male	52		2	7	61
Female	5	12	13	25	55
Total	57	12	15	32	116

Results

Of the 116 strains of *Neisseria,* 61 were isolated from male and 55 from female patients. Most specimens from men were obtained from the urethra; tonsillar and rectal swabs were taken only from known homosexuals. Seven of the 61 strains were isolated from the tonsils and two from the rectum. Of the 55 strains isolated from female patients, five were obtained from the urethra, 12 from the cervix, 25 from the tonsils, and 13 from the rectum (table I).

IDENTIFICATION PROCEDURES

Sugar fermentation reaction

The sugar fermentation reaction could be evaluated in only 86 (74%) of the 116 strains; 15 strains could not be subcultured, owing to growth failure, and another 15 strains were contaminated, so that the overgrowth made evaluation impossible (table II).

IF and coagglutination tests

The results of the IF and CoA tests, irrespective of the results of the sugar fermentation reactions, correlated for 95·7% of strains; 80 strains gave a positive result and 31 a negative result with both the

TABLE II *Comparison of results with direct immunofluorescence, coagglutination, and sugar degradation tests*

		Results of sugar degradation			
Test results	*No of strains*	*Dextrose*	*Dextrose and maltose*	*Contaminated*	*Subculture failed*
Positive to					
IF and CoA	80	63	0	9	8
IF only	3	1	0	1	1
CoA only	2	1	0	1	0
Negative to					
IF and CoA	31	1	20	4	6
Total	116	66	20	15	15

IF = indirect immunofluorescence
CoA = coagglutination

IF and CoA tests. The five conflicting results were found in one strain isolated from the urethra of a male patient, in three strains isolated from the rectum, and in one strain isolated from the tonsils. Results thus agreed in 68 (98·6%) of 69 strains isolated from genital specimens, in 31 (96·9%) of 32 strains isolated from tonsillar specimens, and in 12 (80%) of 15 strains isolated from rectal specimens.

Of the 66 strains which had been identified as *N gonorrhoeae* by the isolated dextrose fermentation reaction, 64 showed a positive result to the IF test but two strains did not react. The CoA test confirmed the identification of *N gonorrhoeae* in 64 (97%) of 66 strains.

Discussion

Our results clearly show the difficulties met with in the routine venereological laboratory. The specific identification of *N gonorrhoeae* by its ability to ferment sugar in the subcultures is difficult as well as time-consuming and expensive. Its use is further limited by possible contamination and death of subcultures as well as by the occasional strains which have a decreased ability to degrade sugar. These difficulties are often stressed in published reports but actual numbers of failures are not mentioned.[27]

The direct IF method has proved to be reliable for identification.[16 17] The specificity of the commercially available conjugate is sometimes disputed.[28 29] With practice, however, the method is highly specific compared with the fermentation reaction. The IF method reduces reporting time by at least 24 hours, and the actual technique and the evaluation take no longer than two hours to perform. The method, however, requires skill, experience, and the technical equipment for fluorescent microscopy.

On the other hand, the CoA method does not require specialised technical equipment; the test kit is commercially available. The test takes only a few minutes to perform, is easy to interpret, and is also reproducible. Even dead organisms still show a reaction. The costs of the reagents are similar to those of other methods. In the present study the results of the IF and CoA tests correlated for 111 (95·7%) of the 116 strains tested.

This agreement is even higher when only strains from genital specimens (98·4%) and from pharyngeal specimens (96·9%) are compared with each other. Three of 15 strains isolated from the rectum showed divergent results. Compared with sugar fermentation as the method of reference, there was a similarly high correlation between the IF and CoA tests. Of 86 strains which could be evaluated for their ability to degrade sugar, 66 showed an isolated reaction to dextrose and of these, 63 showed positive reactions to both the IF and CoA tests. Twenty strains which fermented dextrose and maltose all gave negative results with the IF and CoA tests. Of the five strains with divergent results to the IF and CoA tests, one strain with a positive IF and a negative CoA result and one strain with both a negative IF and CoA result showed an isolated dextrose fermentation. These results cannot be interpreted, since a false result to the IF as well as to the CoA test, and the fact that a strain of *N meningitidis* is unable to ferment maltose, may be responsible.[30] Further investigations, such as serological grouping of meningococci, were not carried out.

Menck[23] reported pseudo-CoA (autoagglutination) results to the Phadebact® Gonococcus Test with colonies of *Neisseria* grown on serum-containing media and very rarely on serum-free media. We used the serum-free media, Orion, throughout and had no pseudo-CoA results.

In the routine venereological laboratory investigations must aim at a rapid accurate diagnosis—either by identification or by exclusion of *N gonorrhoeae*—and be inexpensive in time and materials, avoiding subcultures if possible. The present study shows that the sugar fermentation reaction does not fulfil these aims. On the other hand, the IF method confirmed 97% of *N gonorrhoeae* strains identified by sugar fermentation, the CoA yielding the same percentage. The agreement between the CoA and IF tests of 95·7% is also very high. Both methods showed no false-positive results for *N meningitidis*. The CoA test has therefore the same high specificity as the IF test but has the advantage of being a simple and rapid technique.

References

1. Thayer DJ, Martin JE. Improved medium selective for cultivation of *N gonorrhoeae* and *N meningitidis*. *Pub Health Rep* 1966;**81**:559-62.
2. Hollis DG. Sources of *Neisseria lactamica*. *Lancet* 1973;**1**:1010.
3. Faur YC, Weisburg MH, Wilson ME. Isolation of *Neisseria meningitidis* from the genitourinary tract and anal canal. *J Clin Microbiol* 1975;**2**:178-82.
4. Chapel TA, Gotewood C, Keane MB. *Neisseria meningitidis* in the anal canal of homosexual men. *J Infect Dis* 1977;**136**:810-2.
5. Givan KF, Thomas BW, Johnston AG. Isolation of *Neisseria meningitidis* from the urethra, cervix and anal canal: further observations. *Br J Vener Dis* 1977;**53**:109-12.
6. Willcox RR, Spencer RC, Ison C. Which *Neisseria*? *Br J Vener Dis* 1977;**53**:394-7.
7. Dunn WA, Gordon MH. Clinical and bacteriological aspects of an epidemic simulating influenza. *Br Med J* 1905;**3**:421-7.
8. Lingelsheim W von. Die bakteriologischen arbeiten der königlichen hygienischen station zu beuthen oberschlesien während der genickstarreepidemie in Oberschlesien im winter 1904/5. *Klin Jahrbuch* 1906;**15**:373-488.
9. Thayer JD. Gonorrhea. In: Bodily HL, Updyke EL, Mason JO, eds. *Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections*. 5th ed. New York: American Public Health Association Inc, 1970:281-99.

10. Kellogg DS, Turner EM. Rapid fermentation confirmation of *Neisseria gonorrhoeae*. *Appl Microbiol* 1973;**25**:550-2.
11. Deacon WE, Peacock WL, Freeman E, Harris A. Identification of *N gonorrhoeae* by means of fluorescent antibodies. *Proc Soc Exper Biol Med* 1959;**101**:322-5.
12. Gallwey JMD, Nicol CS, Ridley M. Immunofluorescent antibody technique in the diagnosis of gonorrhoea by direct smears from the male. *Br J Vener Dis* 1967;**43**:168-9.
13. Henderson RA, Rutherford S, Phelps JA, Robertson P. Experience with a rapid direct immunofluorescent test for the gonococcus as a bench procedure in veneral disease clinics. *Br J Vener Dis* 1970;**46**:205-7.
14. Thin RNT. Immunofluorescent method for diagnosis of gonorrhoea in women. *Br J Vener Dis* 1970;**46**:27-30.
15. Schmid EE, Sonnabend W, Jung M, Krech U. Gonorrhoe oder unspezifische infektion. Differenzierung durch direkte IF. *Schweiz Med Wochenschr* 1971;**101**:1717-24.
16. Rufli Th. Zur IF-Diagnostik der gonorrhoe. *Dermatologica* 1973;**146**:269-80.
17. Reichlin B, Rufli Th. Zur aussagekraft heute verfügbarer gonorrhoe-untersuchungsmethoden. *Schweiz Med Wochenschr* 1974;**104**:1712-7.
18. Tronca E, Handsfield HH, Wiesner PJ, Holmes KK. Demonstration of *N gonorrhoeae* with fluorescent antibody in patients with disseminated gonococcal infection. *J Infect Dis* 1974;**129**:583-6.
19. Jephcott AE, Morton RS, Turner EB. Use of transport and culture medium combined with immunofluorescence for the diagnosis of gonorrhoea. *Lancet* 1974;**2**:1311-3.
20. Hare MJ. Comparative assessment of microbiological methods for the diagnosis of gonorrhoea in women. *Br J Vener Dis* 1974;**50**:437-41.
21. Danielsson D, Kronvall G. Slide agglutination method for the serological identification of *N gonorrhoeae* with anti-gonococcal antibodies adsorbed to protein-A-containing staphylococci. *Appl Microbiol* 1974;**27**:368-74.
22. Kronvall G. A rapid slide agglutination method for typing pneumococci by means of specific antibody absorbed to protein-A-containing staphylococci. *J Med Microbiol* 1973;**6**:187-90.
23. Menck H. Identification of *Neisseria gonorrhoeae* in cultures from tonsillo-pharyngeal specimens by means of a slide coagglutination test (Phadebact® gonococcus test). *Acta Pathol Microbiol Scand* 1976;Sect B **84**:139-44.
24. Barnham M, Glynn AA. Identification of clinical isolates of *Neisseria gonorrhoeae* by a coagglutination test. *J Clin Path* 1978;**31**:189-93.
25. Stacey P, Warner G. Identification of *Neisseria gonorrhoeae* and *Neisseria meningitidis* by a carbohydrate disc technique. *J Clin Path* 1973;**26**:303-4.
26. Martin JE, Lester A. Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. HSMHS 1971;**86**:30-3.
27. Olcén P, Danielsson, D, Kjellander J. Laboratory identification of pathogenic *Neisseria* with special regard to atypical strains: an evaluation of sugar degradation, immunofluorescence and coagglutination tests. *Acta Pathol Microbiol Scand* 1978;Sect B **86**:327-34.
28. Petzoldt D. Die immunofluoreszenz in der gonorrhoe-diagnostik. *Arch Dermatol Forschung* 1972;**244**:288-98.
29. Barthelmes H, Mühler F, Droste KD, Kern A. Die herstellung, testung und anwendung eines artspezifischen, FITC-markierten antigonokokkenserums. *Dermatol Monatsschr* 1973;**159**:929-43.
30. Beno DW, Devine LF, Larson GL. Identification of *N meningitidis* carbohydrate fermentation patterns in Mueller-Hinton broth. *J Bacteriol* 1968;**96**:563-8.

Br J Vener Dis 1980;**56**:148-50.

Prevalence of cervical gonorrhoea in women with unwanted pregnancies

B VAN DER LUGT,* A C DROGENDIJK,† AND J R J BANFFER‡

*From the *Dr W F Stormkliniek, the †Institute of Gynaecology and Obstetrics, Erasmus University, and the ‡Laboratory for Epidemiological Bacteriology, Municipal Public Health Service, Rotterdam, the Netherlands*

SUMMARY Cervical specimens were collected from 1688 women attending a gynaecological clinic for induced abortion between February and December 1978. Thirteen (0·77%) women had positive culture results for *Neisseria gonorrhoeae.* Among those women who had a completed family or who came from outside Rotterdam or from outside the neighbouring Rijnmond area, the isolation rate was very low. None of the women with positive culture results developed salpingitis after abortion; all of them had been treated prophylactically with tetracycline. No reasons appear to exist for considering women who seek abortions to be in a high-risk group for cervical gonorrhoea. Whether or not antibiotic prophylaxis should be given for the prevention of salpingitis following abortion requires further study of potentially pathogenic micro-organisms inhabiting the vagina and cervix of women applying for abortion.

Introduction

Abortion performed in women with cervical gonorrhoea may cause an ascending infection.[1] To our knowledge, there have been no reports of a comprehensive study of the occurrence of this complication, although some abortion clinics routinely prescribe antibiotics to prevent such infection. If such prophylactic treatment is aimed at gonococcal infections, it is important to know the proportion of women harbouring gonococci among those who apply for abortion. For this reason, we collected cervical specimens for culture for *Neisseria gonorrhoeae* from a large group of women in an urban centre in the Netherlands.

Patients and methods

Between February and December 1978 we collected specimens from the cervical canal of 1688 consecutive women living in and around Rotterdam who were applying for abortions at this clinic. Specimens were taken with charcoal-impregnated cottonwool swabs; these were placed in Stuart's transport medium. Within four hours of collection two Thayer-Martin media were inoculated; vancomycin, nystatin, and colistin had been omitted from one of these media. Gonococci were identified according to standard bacteriological methods by Gram staining, oxidase test, and carbohydrate fermentation.

A five-day course of tetracycline was given in the clinic after the induced abortion as routine prophylaxis against ascending infection. Although we instructed all the patients to visit the clinic again 2-3 weeks after abortion, only a few returned. Most of the women living in Rotterdam, however, did so. All patients with positive culture results were followed up.

After 2-8 months the patients' general practitioners were asked whether or not their patient had any evidence of salpingitis, such as fever or abdominal pain.

Results

Demographic details of the patients studied are given in table I. *N gonorrhoeae* was cultured from the cervical canal of 13 (0·77%) women. No woman either having a completed family or living outside Rotterdam and Rijnmond had positive culture results; relatively few positive results were found in married women or in those of 30 years or more.

None of the patients with or without gonorrhoea developed salpingitis.

Address for reprints: Professor A C Drogendijk, Institute for Gynaecology and Obstetrics, Ziekenhuis Dijkzigt, Dr Molewaterplein 40, 3015 GD Rotterdam, the Netherlands

Received for publication 20 June 1979

TABLE I *Demographic details and the prevalence of cervical gonorrhoea in 1688 women with unwanted pregnancies*

	Patients studied		*Positive culture results*	
Demographic details	*No (A)*	*%*	*No (B)*	*% ($\frac{B}{A}\times 100$)*
Residence				
Rotterdam	732	45	10	1·37
Rijnmond (excluding Rotterdam)	114	7	2	1·75
Others	842	48	1	0·12
Age (years)				
<20	340	20	4	1·18
20-29	740	44	7	0·95
>29	608	36	2	0·33
Marital state				
Unmarried	813	48	9	1·11
Married	812	48	4	0·49
Divorced/widow	63	4	0	
Origin				
Dutch	1191	70	9	0·76
Surinam/Dutch Antillian	188	11	3	1·60
Immigrant labourer	147	9	0	
Others	162	10	1	0·62
Indication for abortion				
Completed family	637	38	1	0·16
Other	1051	62	12	1·14
Total	1688	100	13	0·77

Discussion

The results of our study of the prevalence of cervical gonorrhoea in patients applying for abortion agree with those of other studies (table II) with one exception; Burkman and co-workers[1] reported a much higher prevalence. Further information which might allow comparison with the other studies is not given. There is a difference between our study and another Dutch study,[2] as the latter included 80-85% of women of German origin. Whether or not cervical gonorrhoea is common in patients applying for abortion cannot be stated, as there is no information available on this subject.

In a study of cervical gonorrhoea in patients undergoing gynaecological examinations by their general practitioner,[3] of a total of 2000 women 0·4% appeared to have positive results. Although the incidence of genital gonorrhoea in Dutch women has increased by more than 10% in the last three years (Bijkerk H, personal communication), there is no evidence that patients applying for induced abortions should be considered a high-risk group.

On the basis of the results of this study the question whether or not prophylactic antibiotic treatment is useful in induced abortion cannot be answered definitely. An answer can only be given with regard to special categories of the population. Antibiotic prophylaxis did not seem to be justified in older married women or in those visiting our clinic from outside Rijnmond.

Recent published reports[4 5] draw attention to *Chlamydia trachomatis,* which is a tetracycline-sensitive micro-organism and a possible cause of sexually transmitted diseases. *C trachomatis* has recently been shown to cause salpingitis,[6 7] so if antibiotics are prescribed prophylactically they should be antichlamydial as well as antigonococcal.

The authors thank Dr E Stolz, Erasmus University, for his valuable advice during and after the study; Dr J Huisman, Municipal Public Health Service, Rotterdam, for his help in the transport of culture specimens; and technicians of the Dr W F Stormkliniek and the Bacteriological Epidemiological Laboratory for their assistance.

TABLE II *Published reports of the prevalence of cervical gonorrhoea in women with unwanted pregnancies*

Author (date of publication)	*Date of study*	*No of women*	*Country (and city)*	*% Prevalence*
Burkman *et al* (1976)	1973-75	4823	USA (Baltimore)	2·7
Hodgson *et al* (1974)	1972-73	10 453	USA (Minnesota)	0·67
Goldsmith (1974)	1973	560	USA (Alameda)	0·71
Querido (1979)	1978	1021	Netherlands (Utrecht)	0·30
van der Lugt *et al* (1980) (present study)	1978	1688	Netherlands (Rotterdam)	0·77

References

1. Burkman RT, Tonascia JA, Atienza AF, *et al.* Untreated endocervical gonorrhoea and endometritis following elective abortion. *Am J Obstet Gynecol* 1976; **126**: 648-51.
2. Querido L. *Abortus Provocatus en Geslachtsziekte.* The Hague: Vereniging Stimezo Nederland, 1979.
3. Bänffer JRJ, Huisman J, Hennekam Y. De frequentie van. *Neisseria gonorrhoeae, Trichomonas vaginalis* en *Candida albicans* bij 2000 vrouwen te Rotterdam. *Huisarts en Wetenschap* 1975; **18**: 2-7.
4. McCormack WM, Alpert S, McComb DE, *et al.* Fifteen-month follow-up study of women infected with *Chlamydia trachomatis. N Engl J Med* 1979; **300**: 3.
5. Stolz E, Schuller JL. De niet-gonorroïsche urethritis. *Med Jaar* 1979; 380-93.
6. Mårdh PA, Ripa T, Svensson L, *et al. Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1979; **296**: 1377-9.
7. Treharne JD, Ripa KT, Mårdh PA, *et al.* Antibodies to *Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis* 1979; **55**: 26-9.

Cryosurgery: a natural choice.

Recent developments by Spembly have resulted in a range of equipment bringing the benefits of cryosurgery to the treatment of genital warts.

With remarkable results.

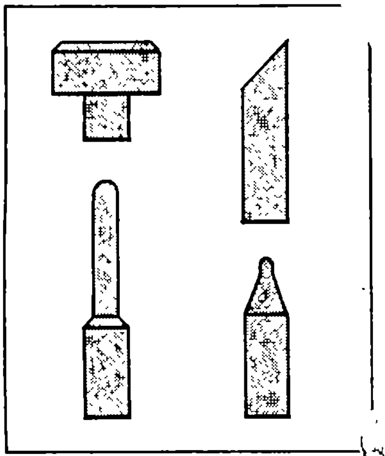
It's a relatively painless, bloodless, approach with a high degree of patient acceptability.

That's why cryosurgery is becoming a natural choice for an ever growing number of physicians.

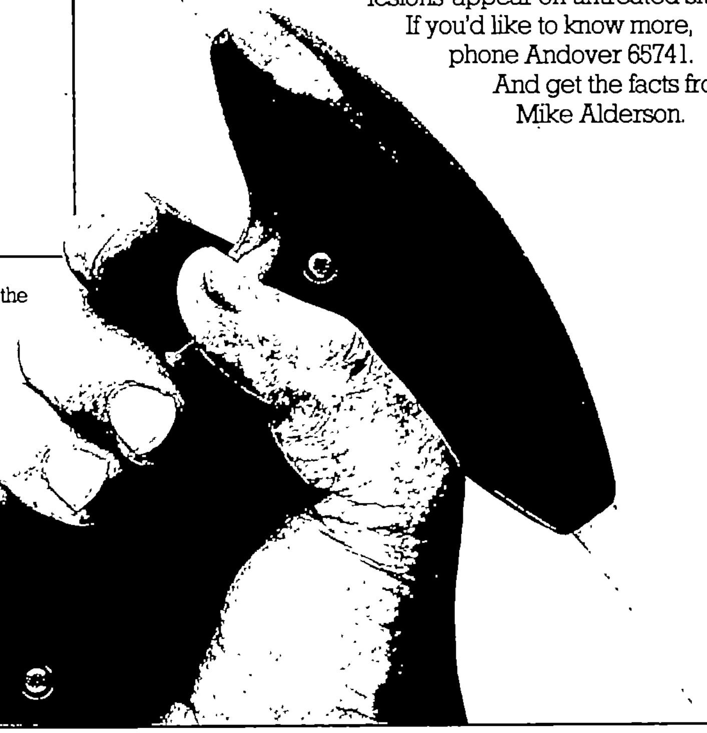
Spembly's range is well suited to the ablation of genital warts, where extensive areas can be treated with no local anaesthetic. The technique is easily repeated should new lesions appear on untreated sites.

If you'd like to know more, phone Andover 65741.

And get the facts from Mike Alderson.

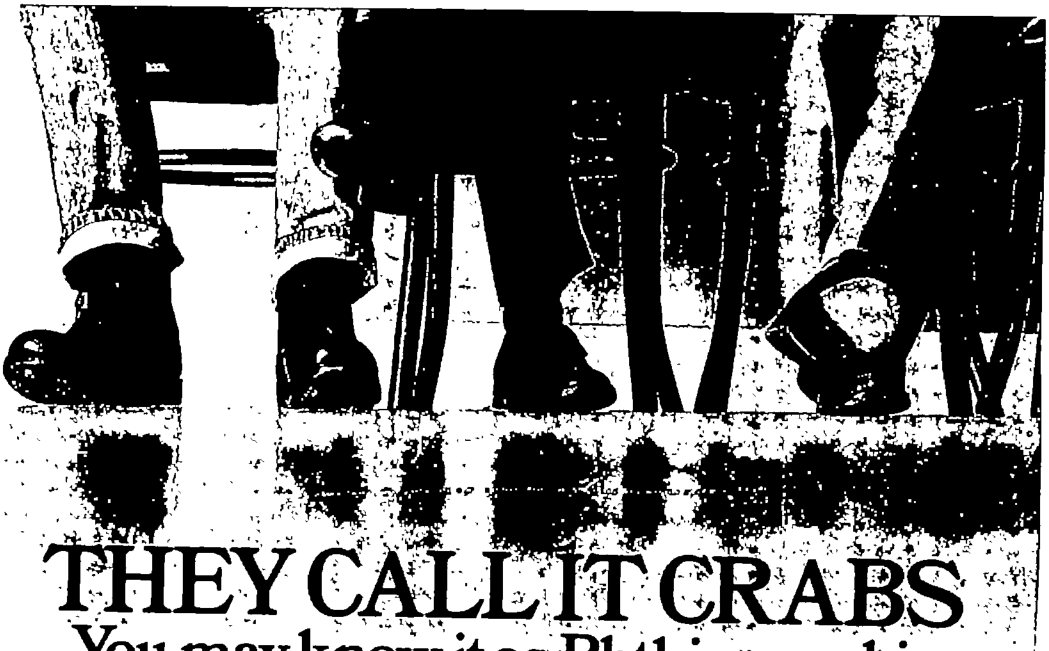


Some of the wide range of interchangeable tips for use with the illustrated Cryogun.



Spembly

Spembly Limited
Newbury Road Andover Hampshire SP10 4DR England
telephone (0264) 65741 telex 47403



THEY CALL IT CRABS
You may know it as Phthirus pubis

Whatever you call them, pubic lice are a problem, because any measure you adopt to eradicate them must take care of both adult lice and nits, while being cosmetically acceptable. Quellada Shampoo fills the bill particularly well. Its active constituent, gamma benzene hexachloride, is rapidly lethal to crab lice, while the foaming shampoo base ensures that Quellada penetrates through to the nits. So a single application of Quellada kills lice *and* nits. Quellada Shampoo is easy and pleasant to use, because it doesn't smell or irritate the skin.

Quellada PC Shampoo
gamma benzene hexachloride 1%

kills pubic lice and nits in 4 minutes.

Presentation
Gamma benzene hexachloride 1% in a specially formulated foaming shampoo base.

Uses
Antiparasitic and ovacidal. For use on hairy areas of the body in the treatment of lice infestations, e.g. head lice, body lice, pubic lice.

Dosage and administration
Apply to wet hair, rub vigorously for four minutes, rinse and comb with fine-tooth comb.

Contra-indications, Warnings etc.
Avoid contact with the eyes. Should lather accidentally get into eyes, flush immediately with water. Do not use more than twice in any one infestation nor in any one week. Do not use on broken or infected skin.

Packaging and price
Bottles of 100 and 500ml. Basic NHS cost 60p for 100ml.

Product Licence No.
0036/5001.

Further information is available on request.

Stafford-Miller Limited,
Professional Relations Division,
Hatfield, Herts. AL10 0NZ.

Br J Vener Dis 1980;**56**:151-5.

Comparative study of bacampicillin and ampicillin in the treatment of uncomplicated gonorrhoea*

PATRICIA J SPENGLER AND LARRY D EDWARDS
From the Biomedical Sciences Department, University of Illinois, Rockford, Illinois, USA

SUMMARY Bacampicillin hydrochloride, a pro-drug ester of ampicillin trihydrate which is hydrolysed to ampicillin after absorption, was used in a randomised comparative study of ampicillin 3·5 g and bacampicillin 1·6 g (each with probenecid 1 g) in the treatment of uncomplicated gonorrhoea. This dose of bacampicillin was selected because in serum it gives approximately the same peak concentration of ampicillin as 3·5 g of the parent drug. Genital, pharyngeal, and anal cultures were performed at the initial visit and at follow up 5-9 days after treatment. There was no statistically significant difference in the microbiological or clinical response of patients with genital gonorrhoea who were treated with ampicillin and those treated with bacampicillin. At 5-9 days after treatment, 93% of the ampicillin-treated patients and 89% of the bacampicillin-treated patients had negative genital, pharyngeal, and anal culture results; furthermore, 87% and 89% respectively had no symptoms of infection. Fewer gastrointestinal side effects were noted in the patients treated with bacampicillin.

Introduction

Oral antibiotic therapy has the advantages of greater patient co-operation and a lower rate of hypersensitivity reactions;[1 2] moreover, there is a need for effective oral antibiotics which have the potential for eradicating gonorrhoea from the pharynx as well as from anogenital sites.

Several investigators have reported high pharyngeal colonisation rates, particularly in women and homosexual men. In studies by Wiesner *et al*[3] and Bro-Jorgensen and Jensen[4] of patients who had positive culture results for *Neisseria gonorrhoeae* at genital, rectal, or pharyngeal sites, pharyngeal colonisation rates ranged from 10-10·3% in women, 3·2-6% in male heterosexuals, and 20·9-25% in male homosexuals.[3 4] In a population known to have infrequent orogenital sexual contact, Noble *et al*[5] found that among patients with concurrent genital or rectal gonorrhoea 3·3% of the men and 1·9% of the women had gonococcal colonisation of the pharynx. In a recent study by Gartner and Edwards (paper presented at 79th Annual Meeting, American Society for Microbiology, Los Angeles-Honolulu, 1979) multiple cultures were obtained from each potential site of infection—pharynx, anus, and genital tract—before treatment. Patients were selected for the study if they had either a positive result to a genital smear or a previous positive genital culture result. In this study 23·8% (10/42) of heterosexual women and 8·9% (4/45) of heterosexual men who were culture-positive at a genital or an anal site also had a positive result to a pharyngeal culture.

Bro-Jørgensen and Jensen[4] and Karney *et al*[6] demonstrated that *N gonorrhoeae* is difficult to eradicate from the pharynx with the multiple antibiotic regimens that they tried. The Centre for Disease Control in Atlanta, Georgia, does not recommend the use of the standard oral regimen for treating genital gonorrhoea (ampicillin 3·5 g with probenecid 1 g) for the treatment of pharyngeal gonorrhoea.[7] However, in a recent study by Di Caprio[8] success rates of 96·1-98·7% in the treatment of pharyngeal gonorrhoea were achieved with ampicillin trihydrate 3·5 g with probenecid 1 g initially, followed by 500 mg four times daily for two days, a total of 7·5 g of ampicillin.

In view of the need for oral antibiotics which can eradicate pharyngeal as well as genital gonorrhoea, we decided to study bacampicillin hydrochloride, an oral preparation which is a pro-drug ester of ampicillin trihydrate. Bacampicillin, after absorption, is hydrolysed to ampicillin and gives serum concentrations two to three times higher than an equimolar dose of ampicillin trihydrate.[9 10]

---

*This study was presented in part at the 79th Annual Meeting of the American Society for Microbiology, Los Angeles-Honolulu, May 1979

Address for reprints: Dr L D Edwards, Biomedical Sciences Department, Division of Infectious Diseases, Rockford School of Medicine, 1601 Parkview, Rockford, Illinois 61101, USA

Received for publication 21 August 1979

Patients and methods

STUDY POPULATION

Sixty-one men and 22 women with culture-positive uncomplicated gonorrhoea were randomly assigned to one of two oral treatment groups. One group was treated with ampicillin 3·5 g, the other with bacampicillin 1·6 g, a one-third equimolar dose. Probenecid 1 g was administered simultaneously with each drug.

The patients were volunteers aged 18 years or more, who attended the sexually transmitted diseases clinic at the Winnebago County Department of Public Health, Rockford, Illinois, USA. Those patients who were pregnant or nursing an infant, wished to donate blood during the study period, were known or suspected to be allergic to penicillin or cephalosporins, had hepatic disease or impaired renal function, required other concomitant antimicrobial therapy, had received systemic antimicrobial therapy within the previous month, or had infectious mononucleosis, impaired immunological function, syphilis, or chronic urinary tract infection were excluded from the study.

COLLECTION OF SPECIMENS

At the initial visit, urethral and endocervical specimens were obtained by calcium alginate swabs from men and women respectively for Gram stain and culture. Pharyngeal and anal specimens were obtained for culture from all patients. To monitor adverse drug reactions, total blood counts, urine analyses, and serum analyses for hepatic and renal function (bilirubin, alkaline phosphatase, creatinine, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, and urea nitrogen) were performed. Routine serological Venereal Disease Research Laboratory (VDRL) tests for syphilis were performed; patients with reactive VDRL test results were excluded from the study. Gram stains, freshly centrifuged urine sediment specimens, and saline-diluted wet-mount preparations of vaginal secretions were examined microscopically for yeast forms and trichomonads. Patients with apparent candidosis or trichomoniasis were not included in the study. Two hours after treatment, specimens of urine, blood, and saliva were collected for determination of ampicillin concentrations.

Follow-up visits took place 5-9 days after treatment, at which time patients were questioned about symptoms and possible drug-related reactions. Gram stains, cultures, wet-mount preparations, urine analyses, and tests to monitor for adverse drug reactions were repeated as for the initial visit. The procedures followed were in accordance with the ethical standards of the committees on human experimentation of the University of Illinois.

DIAGNOSIS

All cultures were inoculated directly on to modified Thayer-Martin medium (Baltimore Biological Laboratories, Cockeysville, Maryland) containing vancomycin, colistin, nystatin, and trimethoprim[11] and were incubated immediately at 35°C in 5-10% carbon dioxide. Cultures were transported to the Rockford School of Medicine laboratories where standard procedures for isolation and identification of *N gonorrhoeae* were followed with confirmation of pharyngeal and anal isolates by sugar fermentation tests.[12]

ANTIBIOTIC SENSITIVITIES

Minimum inhibitory concentrations (MICs) of ampicillin for *N gonorrhoeae* isolates were determined by the agar dilution technique.[13] A suspension of 10^5 organisms per ml in Mueller-Hinton broth supplemented by 1% IsoVitaleX (Baltimore Biological Laboratories, Cockeysville, Maryland) was delivered by automatic inoculator to the surface of each agar plate with antibiotic concentrations from 0·049 to 100·0 μg/ml. The ampicillin-containing agar plates were prepared just before use in GC medium base (Difco Laboratories, Detroit, Michigan) with 1% IsoVitaleX. The MIC was defined as the dilution with no visible growth after 48 hours of incubation.

MICs of penicillin were not determined.

β-LACTAMASE PRODUCTION

Fresh subcultures of *N gonorrhoeae* on chocolate agar were tested for β-lactamase production using the chromogenic cephalosporin method.[14]

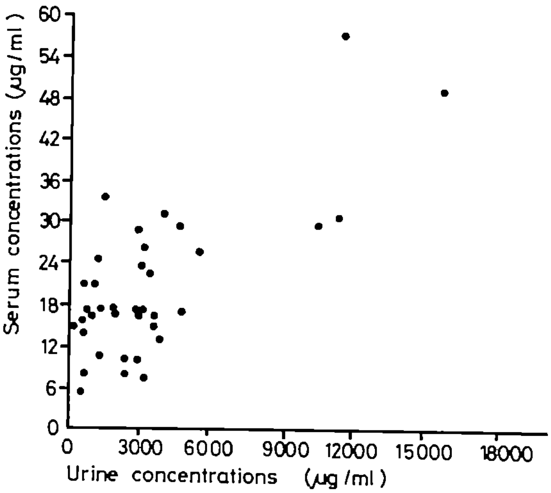


FIG 1 *Correlation of ampicillin concentrations in serum and urine specimens from 37 patients two hours after administration of ampicillin 3·5 g with probenecid 1 g.*

ANTIMICROBIAL ASSAY FOR AMPICILLIN CONCENTRATIONS

Specimens of urine, blood, and saliva were frozen immediately and transported to the Rockford School of Medicine laboratories, where they were stored at −20°C. Specimens were tested for drug concentrations within 72 hours of collection by agar-well bioassay[15] using *Bacillus subtilis* as the indicator organism.

STATISTICAL METHODS

χ^2 analysis with Yates's correction was used for statistical comparisons of proportions and Student's *t* test for unpaired data (one-tailed) for comparisons of means. Correlation analyses (figs 1 and 2) were computed by Pearson product-moment correlation coefficients using the one-tailed Student's *t* test for unpaired data to determine levels of significance.

Results

PATIENT POPULATION

Of the 83 patients treated, 57 (69%) returned for a follow-up visit. The treatment groups were statistically similar with regard to sex, race, history of previous infection, and age (table I).

The study population differed from our regular clinic population in that it was predominantly male whereas the latter was equally divided between men and women. More men than women volunteered for the study, and women who were possibly pregnant were excluded. Racial distribution was representative of the regular clinic population; both the study group and the regular clinic population were approximately 50% white and 50% non-white.

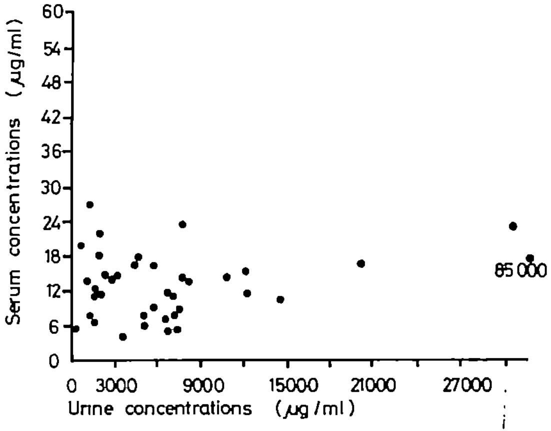


FIG 2 *Correlation of ampicillin concentrations in serum and urine specimens from 38 patients two hours after administration of bacampicillin 1·6 g with probenecid 1 g.*

TABLE I *Characteristics of patients treated for gonorrhoea with ampicillin and bacampicillin*

	Treatment group	
Characteristics	*Ampicillin (n = 30)*	*Bacampicillin (n = 27)*
Sex		
Male	21	22
Female	9	5
Race		
White	14	11
Non-white	16	16
Previous gonococcal infection	14	15
Mean age (years)	24·1	23·9

Two men in the study group stated that they were homosexuals and one stated that he was bisexual; the rest of the patients claimed to be practising heterosexuals.

GRAM STAINS AND CULTURES

At the time of the initial visit, intracellular Gram-negative diplococci typical of *N gonorrhoeae* were identified on Gram stains of urethral exudate of all 43 men and on Gram stains of endocervical exudate of 11 (79%) of the 14 women. Three women who entered the study as named contacts of men with confirmed gonococcal urethritis had negative Gram stains and positive endocervical culture results.

Initially, all patients had positive genital culture results and negative pharyngeal culture results. Only one patient, female, had a positive result to an anal culture.

MICs

MICs for all 58 isolates taken at the initial visit are shown in table II. There was no significant difference in the distribution of MICs between the group treated with ampicillin and that treated with bacampicillin ($\chi_4^2 = 3\cdot63$, $P \cong 0\cdot46$).

TABLE II *Minimum inhibitory concentrations of ampicillin for 58 isolates of Neisseria gonorrhoeae obtained at patients' initial visits*

	Isolates from patient-groups					
	Ampicillin (n = 31)		*Bacampicillin (n = 27)*		*Total*	
MICs (μg/ml	*No*	*%*	*No*	*%*	*No*	*%*
0·049	10	32·2	12	44·4	22	37·9
0·098	8	25·8	6	22·2	14	24·1
0·195	3	9·7	5	18·5	8	13·8
0·390	9	29·0	4	14·8	13	22·4
0·780	1	3·2	0	0	1	1·7
Mean	0·104		0·086		0·170	
Median	0·198		0·137		0·098	

β-LACTAMASE PRODUCTION

No isolates produced β-lactamase.

DRUG CONCENTRATIONS

For 37 patients treated with ampicillin (fig 1), the serum ampicillin concentrations at two hours ranged from 5·8-58·0 μg/ml with a median value of 18·3 μg/ml and a mean value of 21·1 μg/ml; urine concentrations ranged from 370·0-15 400 μg/ml with median and mean values of 2595 and 3364·6 μg/ml respectively.

For 38 patients treated with bacampicillin (fig 2), the serum ampicillin concentrations at two hours ranged from 4·7-28 μg/ml with median and mean values of 13·6 μg/ml; urine concentrations ranged from 700-85 000 μg/ml with median and mean values of 5220 μg/ml and 8292·5 μg/ml respectively.

No ampicillin was detected in saliva specimens from patients in either treatment group.

There was a strong positive correlation between serum and urine concentrations in the ampicillin-treated patients ($r = 0\cdot72$, $\mathrm{P} < 0\cdot001$). Concentrations of ampicillin in the serum and urine of bacampicillin-treated patients showed no significant correlation ($r = 0\cdot18$, $\mathrm{P} \hat{=} 0\cdot14$).

Serum drug concentrations at two hours in the ampicillin-treated patients were significantly higher than those in the bacampicillin-treated group ($t = 3\cdot70$, $\mathrm{P} < 0\cdot001$) and urine concentrations were significantly lower ($t = -2\cdot10$, $\mathrm{P} < 0\cdot025$).

MICROBIOLOGICAL RESPONSE

The microbiological response was similar in the two groups ($\chi^2_1 = 0\cdot02$, $\mathrm{P} \hat{=} 0\cdot9$). Ninety-three per cent (28/31) of the ampicillin-treated patients and 89% (24/27) of the bacampicillin-treated patients had negative culture results at follow up, including the female patient whose initial anal culture gave a positive result. Five heterosexual patients, four male and one female, who were initially culture-positive at genital sites, were culture-positive 5-9 days after treatment. Four had positive results at genital sites; one male patient had a positive pharyngeal culture result after treatment although his initial pharyngeal culture had a negative result. On examination and interview during the follow-up visit, all five of these patients were asymptomatic.

For the five patients who were culture-positive after treatment, no significant difference was noted between MICs on initial and post-treatment isolates (table III).

The four men, including the one with a positive pharyngeal culture result, admitted to further contact during the study period and may represent reinfections rather than treatment failures.

Gram stains gave positive results for the three men with positive urethral culture results after treatment. These three patients, two initially treated with bacampicillin and one treated with ampicillin, and the man with a positive pharyngeal culture result, who was initially treated with bacampicillin, responded satisfactorily to retreatment with intramuscular spectinomycin or aqueous procaine penicillin G. The culture-positive female patient had a negative Gram-stain result and therefore was not immediately retreated. After culture results were known, we were unable to locate this patient.

TABLE III *MICs of ampicillin for N gonorrhoeae isolates from five patients at initial visit and at follow up (before and after treatment)*

			MIC (μg/ml)	
Patient No	*Sex*	*Drug*	*Initial visit*	*Follow-up visit*
1	M	Bacampicillin	0·390	0·780
2	M	Ampicillin	0·195	0·390
3	M	Bacampicillin	0·049	0·049
4	F	Ampicillin	0·098	0·390
5	M	Bacampicillin	0·390	0·390*

*Pharyngeal isolate

CLINICAL RESPONSE

There was no statistical difference between the treatment groups in clinical response at 5-9 days ($\chi^2_1 = 0\cdot02$, $\mathrm{P} \hat{=} 0\cdot9$). Eighty-seven per cent (26/30) of the patients treated with ampicillin and 89% (24/27) of those treated with bacampicillin had no symptoms at follow up. Seven patients (13·3% of those treated with ampicillin and 11·1% of those treated with bacampicillin) had symptoms after treatment, all of whom were culture-negative. Of these, four were women who complained of vaginal discharge; one had candidosis, one trichomoniasis, and two unexplained vaginal discharge. Three men had either dysuria, discharge, or frequency of micturition.

ADVERSE REACTIONS

Adverse reactions occurred more commonly in the ampicillin group (23·3%; 7/30) than in the bacampicillin group (3·7%; 1/27) ($\chi^2_1 = 3\cdot06$, $\mathrm{P} \hat{=} 0\cdot08$). Five patients had diarrhoea with four to six stools per day for one or two days; two had mild nausea or epigastric discomfort. One patient treated with ampicillin experienced globus hystericus. Haematological tests, urine analyses, and tests for renal and hepatic function showed no drug-related abnormalities.

Discussion

Studies have shown that a one-third equimolar dose of bacampicillin (1·6 g) results in approximately the same peak serum concentrations as ampicillin 3·5 g and that peak ampicillin concentrations are obtained approximately 30 minutes earlier with bacampicillin than with ampicillin.[9,10]. Unpublished information supplied by the pharmaceutical company (Pfizer Inc., New York, NY) indicated that peak serum concentrations of ampicillin would be obtained at 1·5 hours with bacampicillin and 2·2 hours with ampicillin when these drugs are administered without probenecid. We were limited to collecting single specimens for drug assays in this outpatient clinic and all our patients received probenecid; thus we chose to collect specimens at two hours. The significantly lower serum concentrations and higher urine concentrations obtained in the bacampicillin-treated group suggest that peak serum concentrations occurred earlier in the bacampicillin group than in the ampicillin group.

There was no statistical difference in microbiological response or clinical response between the two treatment groups. Bacampicillin in the dosage used was as effective as the standard regimen of treatment with ampicillin.

The persistence of symptoms in 13% (7/52) of patients who were culture-negative for *N gonorrhoeae* after treatment suggests that they were initially infected with multiple micro-organisms. Although we did not perform cultures for *Chlamydia trachomatis,* Bowie *et al*[16] and Holmes *et al*[17] isolated *C trachomatis* from 18% and 19% respectively of men with urethral gonorrhoea, all of whom developed postgonococcal urethritis.

Other micro-organisms have also been implicated as causative agents of nongonococcal urethritis, cervicitis, and vaginitis. In two of the four women with persistent symptoms, candidosis and trichomoniasis were diagnosed at 5-9 days although the presence of yeasts or trichomonads was not noted before treatment.

With the doses used in this study, we were unable to detect ampicillin in the saliva of patients from either treatment group. We could not evaluate the efficacy of bacampicillin in the treatment of pharyngeal gonorrhoea since our study population mainly comprised male heterosexuals, none of whom had pharyngeal colonisation before treatment. The administration of bacampicillin 1·6 g with probenecid 1 g, however, was tolerated with minimal gastrointestinal side effects and was as effective as the standard oral regimen of ampicillin trihydrate for anogenital gonococcal infection.

Since Di Caprio *et al*[8] found ampicillin to be effective in treating pharyngeal gonorrhoea when using a dose of 3·5 g with probenecid 1 g followed by 500 mg four times daily for two days and we found bacampicillin 1·6 g to be as effective as the single dose ampicillin-probenecid regimen in genital gonorrhoea, we believe that bacampicillin should be studied at higher doses in a population with a high frequency of orogenital sexual practices to see if adequate salivary concentrations can be obtained and correlated with eradication of *N gonorrhoeae* in the pharynx.

Grant support was given by Pfizer Pharmaceuticals, New York, USA.

References

1. Finland M, Weinstein L. Complications induced by antimicrobial agents. *N Engl J Med* 1953;**248**:220-6.
2. Weinstein L. The complications of antibiotic therapy. *Bull NY Acad Med* 1955;**31**:500-18.
3. Wiesner PJ, Tronca E, Bonin P, Pedersen AHB, Holmes KK. Clinical spectrum of pharyngeal gonococcal infection. *N Engl J Med* 1973;**288**:181-5.
4. Bro-Jørgensen A, Jensen T. Gonococcal pharyngeal infections: report of 110 cases. *Br J Vener Dis* 1973;**49**:491-9.
5. Noble RC, Cooper RM, Miller BR. Pharyngeal colonisation by *Neisseria gonorrhoeae* and *Neisseria meningitidis* in black and white patients attending a venereal disease clinic. *Br J Vener Dis* 1979;**55**:14-9.
6. Karney WW, Turck M, Holmes KK. Comparative therapeutic and pharmacological evaluation of amoxycillin and ampicillin plus probenecid for the treatment of gonorrhea. *Antimicrob Agents Chemother* 1974;**5**:114-8.
7. Wiesner PJ. Gonococcal pharyngeal infection. *Clin Obstet Gynecol* 1975;**18**:121-9.
8. Di Caprio JM, Reynolds J, Frank G, Carbone J, Nishimura R. Ampicillin therapy for pharyngeal gonorrhea. *JAMA* 1978;**239**:1631-3.
9. Magni L, Sjöberg B, Sjövall J, Wessman J. Clinical pharmacological studies with bacampicillin. In: Williams JD, Geddes AM, eds. *Chemotherapy: Penicillins and Cephalosporins,* Vol 5. New York: Plenum Press, 1976;109-14.
10. Sjovall J, Magni L, Bergan T. Pharmacokinetics of bacampicillin compared with those of ampicillin, pivampicillin, and amoxycillin. *Antimicrob Agents Chemother* 1978;**13**:90-6.
11. Martin JE, Armstrong JH, Smith PB. New system for cultivation of *Neisseria gonorrhoeae. Appl Microbiol* 1974;**27**:802-5.
12. Kellogg DS. *Neisseria gonorrhoeae* (gonococcus). In: Lennette E, Spaulding E, Truant J, eds. *Manual of Clinical Microbiology.* Washington DC: American Society for Microbiology, 1974:124-9.
13. Thornsberry C, Gavan TL, Gerlach EH. New developments in antimicrobial agent susceptibility testing. In: Sherris JC, ed. *Cumitech 6.* Washington DC: American Society for Microbiology, 1977:1-13.
14. O'Callaghan C, Morris A, Kirby S, Shingler A. Novel method for detection of β-lactamases by using a chromogenic cephalosporin substrate. *Antimicrob Agents Chemother 1972;*1:283-8.
15. Winters RE, Litwack KD, Hewitt WL. Relation between dose and levels of gentamicin in blood. *J Infect Dis* 1971;**124** (suppl):90-5.
16. Bowie WR, Alexander ER, Holmes KK. Etiologies of postgonococcal urethritis in homosexual and heterosexual men. *Sex Transm Dis* 1978;**5**:151-4.
17. Holmes KK, Handsfield HH, Wang SP, *et al.* Etiology of nongonococcal urethritis. *N Engl J Med* 1975;**292**:1199-205.

Br J Vener Dis 1980;**56**:156-62.

Quantitative aspects of chlamydial infection of the cervix

D HOBSON,* P KARAYIANNIS,* RUTH E BYNG,* ELISABETH REES,†
I ANNE TAIT,† AND JEAN A DAVIES †

*From the Departments of *Medical Microbiology and †Venereology, University of Liverpool, Royal Liverpool Hospital, Liverpool*

SUMMARY In 580 women with *Chlamydia trachomatis* infection of the cervix, the degree of the infection was assessed by counting the number of chlamydial inclusions which developed in McCoy cell monolayers inoculated with cervical swab material under standardised conditions.

In 34% of these women inclusion counts were <100 per monolayer whereas in 36% the counts were ⩾1000. Clinical features in each of these groups were compared to see if certain factors could be identified as the cause, or the result, of high-grade rather than low-grade infection.

A significant association was found between high inclusion counts and the presence of cervical mucopus or cervical ectopy. Oral contraceptives acted additively with ectopy but had no significant effect alone. Concurrent gonorrhoea did not affect the degree of chlamydial infection. High inclusion counts were more common in women under 20 years of age than in older women.

Introduction

Over the past five years, our combined clinical and laboratory investigations of women with genital tract infections with *Chlamydia trachomatis* have shown large variations between individual infected patients, both in the number and severity of clinical signs[1] and in the number of chlamydial inclusions obtained from cervical swabs inoculated into McCoy cell tissue cultures.[2] In a group of 202 sexual contacts of men with nongonococcal urethritis (NGU), women who had cervical mucopus, or those who had cervical ectopy and were also using oral contraceptives, showed a significantly higher prevalence of chlamydial infection than did women without these clinical features.[3] It seemed of interest to determine if these or other clinical factors might be correlated not only with a high rate of infection but with a more severe infection than in women in whom these factors were absent.

In McCoy cell tissue cultures infected with *C trachomatis* only a single cycle of development of the agent occurs, and each intracellular inclusion found after incubation represents a single infective chlamydial particle in the original inoculum. Thus, when standard amounts of material from cervical swabs are inoculated and incubated under constant conditions in coverslip monolayers of McCoy cells the final total number of inclusions found over the whole cell sheet should give a direct quantitative measure of the number of infective chlamydial particles in the cervical secretions of each patient.

The sensitivity and reproducibility of the laboratory method we have used has been investigated previously;[2 4] for example, in 48 McCoy cultures inoculated with a single sample of *C trachomatis* the counts on individual coverslips varied from the mean by a maximum of only ±23%, and, when a second swab was taken seven days after the first swab from 106 untreated infected women, the inclusion counts were of the same magnitude in both swabs in 92·5% of cases. Thus, the wide variations in inclusion counts we had observed between patients might be the result of differing degrees of infection in the cervical mucosa in individual patients.

The aim of this study was to see if certain clinical factors might be caused by, or be the cause of, high-grade rather than low-grade infection. In particular it seemed essential to consider (a) the physical state of the cervix—that is, whether or not mucopus or ectopy were present; (b) hormonal effects on cervical epithelium—that is, whether or not the patient was using oral contraceptives; (c) if chlamydial infection might be enhanced opportunistically by concurrent infections, especially gonorrhoea; and (d) the age of the patient.

Address for reprints: Dr D Hobson, Department of Medical Microbiology, The Duncan Building, Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XW

Received for publication 24 September 1979

Accordingly, clinical and laboratory data were compared in the total of 580 women from whom *C trachomatis* had been isolated in this laboratory between January 1975 and December 1978. This series excludes only women with postpartum infection and those who had recently received antibiotic treatment, but it was not restricted to women with a definite history of sexual contact with men with NGU and did not exclude women with concurrent gonorrhoea.

Patients and methods

STUDY POPULATION

The 580 women consecutively found to have chlamydial infection on laboratory examination were all patients attending the sexually transmitted disease (STD) clinics at the Liverpool Royal Infirmary or the Royal Liverpool Hospital or who had been referred to the Liverpool Women's Hospital from various sources because of suspected genital tract infections. Only women whose infections were first diagnosed in the puerperium, or who had been given chemotherapeutic agents within the previous four weeks, were excluded.

Clinically, the group consisted of 70 women from a consecutive series of sexual contacts of men with NGU, whose clinical findings have been compared with those of contacts without chlamydial infection,[3] together with 49 infected women examined in a consecutive unselected series of patients attending a single STD clinic of the Royal Infirmary on specified days (Davies *et al,* unpublished data).

The remaining 341 women were not seen consecutively in the clinics; in fact, they had often been deliberately selected for laboratory investigation because they did show certain clinical signs of genital infection, or because they were contacts of men with NGU or with gonorrhoea, or for various other reasons which made it important to confirm or exclude chlamydial infection in their differential diagnoses and to assess its severity if present. This element of clinical selection does not affect the proposed correlation of laboratory findings with the clinical features presented by the patients, since the investigation is based on the laboratory's total number of chlamydia-positive specimens, unselected and completed in advance of the clinical data being known. Obviously, the proportion of women with such features as cervical mucopus, cervical ectopy, or concurrent gonorrhoea cannot be taken as comparable with that in clinically unselected patients seen consecutively in studies designed for clinico-epidemiological purposes.[1 3]

CLINICAL EXAMINATION

Examination of the patients' clinical signs was made and recorded in the standardised pattern described earlier.[3]

COLLECTION OF SPECIMENS

Swabs for chlamydial isolation were taken only by medical staff of the clinic experienced in the standardised technique previously described,[5] which ensures that both the contents of the endocervical canal and the whole of any area of ectopic columnar epithelium present are sampled.

Swabs for *Neisseria gonorrhoeae* isolation and investigations for syphilis, trichomoniasis, or yeast infections were performed on all patients as previously described.[3]

LABORATORY INVESTIGATIONS

The procedures for the isolation of *C trachomatis* and subsequent counts of the total number of chlamydial inclusions recovered have been previously described in detail.[2 4-6] Briefly, particular attention was paid to the quantitative reproducibility of the isolation procedure—for example, immediate immersion of the swab into fluid transport medium, rapid transport to the laboratory and inoculation usually 2-4 hours after the swab was taken but occasionally after overnight storage at 4°C, followed by inoculation of a standardised amount of homogenised swab material with centrifuge-assistance on to McCoy cell coverslip monolayers of constant age and cell density. For the first 260 swabs, untreated McCoy cells were used, but cells treated with cycloheximide were used for the final 200 specimens, and both methods were used simultaneously for the remaining 120 specimens. Cycloheximide treatment has been adopted because chlamydial inclusions can be identified more rapidly and easily than in fresh cells; the total number of inclusions recovered was consistently higher in treated cells, but rarely by more than twofold, so that the log frequency distribution of counts did not vary significantly between the two methods.

In all cases, the inclusion counts recorded represent the primary inoculation of the first cervical swab to be taken from each patient during her attendance in the clinic. All swabs were allocated serial laboratory numbers, and isolation and inclusion counting procedures were completed before the patient's identity and clinical features were reviewed in the laboratory.

STATISTICAL METHOD

The χ^2 test was used for statistical comparison.

Results

INCLUSION COUNTS AND CLINICAL FEATURES

The wide range of inclusion counts found on culture of the primary cervical swabs for the whole group of

TABLE I *Distribution of chlamydial inclusion counts and clinical factors in 580 women with C trachomatis infection of the cervix*

		% of women with positive clinical factor*				
iwc	*No of women*	*Mucopus*	*Ectopy*	*OC*	*Gonorrhoea*	*Aged <20 years*
<10	84	36	58	49	38	23
10+	112	36	63	50	35	24
100+	178	44	67	54	44	26
1000+	133	69	79	62	44	35
10 000+	73	66	75	66	34	40
Total No of women	580	290	402	306	232	168

iwc = No of inclusions per whole coverslip McCoy culture inoculated with cervical swab material
OC = Oral contraceptives
*In each inclusion-count group

580 women is shown in table I. Although the counts ranged from only one inclusion per whole coverslip (iwc) to >75 000 they showed a substantially normal distribution with a median count of 450 inclusions; 476 (85·5%) of the swabs yielded >10 iwc and 207 (36%) gave ≥1000 iwc.

The distribution of the main clinical factors is also outlined in table I. The overall incidence of mucopus, ectopy, and gonorrhoea was high, as was to be expected by the clinical selection of these women. Nevertheless, certain clinical factors were not uniformly distributed among the whole group.

The greater the inclusion count, the greater was the proportion of patients who were under 20 years of age, showed cervical ectopy or mucopus, or were taking oral contraceptives. There did not however seem to be any significant correlation between the height of the inclusion count and concurrent gonorrhoea.

These observations suggest that the degree or severity of infection in the cervical mucosa varied according to the presence or absence of certain clinical factors affecting the cervix. To evaluate their interactions more precisely the clinical features of women at the opposite ends of this frequency distribution of inclusion counts—that is, those with low counts of <100 iwc and those with high counts of ≥1000—were compared (table II).

TABLE II *Distribution of clinical factors in relation to low or high inclusion counts*

		No of women with inclusion counts		
Clinical factor		*Low (<100 iwc)*	*High (>1000 iwc)*	*Total*
Mucopus	+	70	140	210
	−	126	67	193
Gonorrhoea	+	64	79	143
	−	132	128	260
Ectopy	+	120	160	280
	−	76	47	123
Oral contraception	+	94	130	224
	−	102	77	179
Age (years)	<20	38	76	114
	>20	158	131	289
Total (%)		196 (34)	207 (36)	403

+ Present − absent
iwc = inclusions per whole coverslip (10 mm diam)

Cervical mucopus/concurrent gonorrhoea

The amount of chlamydial growth in the cervical mucosa was significantly greater in women with mucopus than in those without (table II; $\chi^2_1 = 41{\cdot}1$, P<0·001). A heavy chlamydial infection could be the actual cause of the mucopus but alternatively some other clinical factor could be the cause of both the mucopus and the high chlamydial count. For the latter reason, the fact that 143 (35%) of the 403 women had gonorrhoea concurrently with their chlamydial infection had to be taken into account. Gonorrhoea itself is obviously a common cause of cervical mucopus and chlamydial infection might possibly be enhanced opportunistically in tissues already being damaged by *N gonorrhoeae*.

However, high chlamydial counts were not significantly more frequent in women with associated gonorrhoea than in women with chlamydial infection alone ($\chi^2_1 = 1{\cdot}33$, P>0·1) (table II). Furthermore, the

TABLE III *Prevalence of cervical mucopus in relation to inclusion counts in women with or without concurrent gonorrhoea*

	No of women with inclusion counts		
Clinical factor	*Low (<100 iwc)*	*High (>1000 iwc)*	*Total*
Gonorrhoea −			
Mucopus +	45	82	127
Mucopus −	87	46	133
Gonorrhoea +			
Mucopus +	25	58	83
Mucopus −	39	21	60
Total	196	207	403

+ Present − absent
iwc = inclusions per whole coverslip (10 mm diam)

association between mucopus and high chlamydial counts was not dependent on a concomitant infection with *N gonorrhoeae*, as was shown by evaluating separately the data in the groups of women with and without gonorrhoea (table III).

Mucopus was present in a high proportion of all the women studied (210/403, 52%); this could be partly due to the clinical selection of the cases before referral for laboratory diagnosis. However, the proportion of cases with mucopus was similar in women with chlamydial infection alone (127/250, 65%) and in chlamydia-positive women with concurrent gonorrhoea (83/143, 70%) ($\chi^2_1 = 3{\cdot}13$, $P>0{\cdot}05$). Moreover, there was a significant association between the presence of mucopus and high inclusion counts whether or not the woman had a chlamydial infection alone ($\chi^2_1 = 23{\cdot}36$, $P<0{\cdot}001$) or concomitant gonorrhoea ($\chi^2_1 = 17{\cdot}14$, $P<0{\cdot}001$).

Cervical ectopy/oral contraceptives

When these clinical factors were considered separately (table II) each showed a significant association with the height of the inclusion count. There was little difference between simple ectopy or hypertrophic ectopy in relation to inclusion counts and both have been combined for statistical analysis. Because of the clinical selection cervical ectopy was present in over two-thirds of the 403 women studied (table II). However, 57% of these women had high inclusion counts compared with 38% of women without ectopy ($\chi^2_1 = 12{\cdot}3$, $P<0{\cdot}001$). Oral contraceptives were being used by 56% of the 403 women and 58% of these patients had high inclusion counts compared with 43% of women not taking oral contraceptives ($\chi^2_1 = 8{\cdot}98$, $P<0{\cdot}01$).

However, cervical ectopy and oral contraceptives were obviously not independent variables, since only 106 (38%) of the 280 women with ectopy were not taking oral contraceptives and only 50 (22%) women using oral contraceptives did not have cervical ectopy. In fact, the association between the use of oral contraceptives and the presence of ectopy was highly significant ($\chi^2_1 = 47{\cdot}08$, $P<0{\cdot}001$); thus these two clinical factors probably interact in their effect on the height of the inclusion count, as they have previously been shown to affect the incidence of chlamydial infection.[3] To assess this interaction, the four possible combinations of the two factors were further analysed (table IV):

1. In patients without cervical ectopy, high counts were found in 32% of women not taking, and in 48% of women taking, oral contraceptives. The effect of oral contraceptives alone was not significant ($\chi^2_1 = 3{\cdot}42$, $P>0{\cdot}05$).
2. In patients with cervical ectopy, high counts were found in 51% of women not taking, and in 61% of women taking, oral contraceptives. The effect of oral contraceptives alone was not significant ($\chi^2_1 = 2{\cdot}67$, $P>0{\cdot}1$).
3. In patients with oral contraceptives, high counts were found in 48% of women without ectopy and in 61% of women with ectopy. The effect of ectopy alone in these women was not significant ($\chi^2_1 = 2{\cdot}66$, $P>0{\cdot}1$).
4. In patients without oral contraceptives, high counts were found in 32% of those without ectopy and in 51% of those with ectopy. This effect of ectopy alone was significant ($\chi^2_1 = 6{\cdot}67$, $P = 0{\cdot}01$).

When the patients with neither ectopy nor oral contraceptives were compared with those with both ectopy and oral contraceptives, the difference in the proportion of cases with high inclusion counts, 32% and 61% respectively, was significant ($\chi^2_1 = 17{\cdot}8$, $P<0{\cdot}001$).

These results suggest that ectopy and oral contraceptives acting jointly are likely to be associated with high chlamydial counts and that ectopy alone can result in high counts even if the ectopy has not been produced by, or the cervical mucosa has not been acted upon by, oral contraceptives. The effect of oral contraceptives on chlamydial counts appears to be indirect by way of its capacity to produce ectopy. When these possible effects are discounted, by dividing the women into groups (table IV), oral contraceptives appear to have no significant direct effect on the degree of chlamydial infection.

TABLE IV *Interaction of cervical ectopy, oral contraceptives, and inclusion counts*

Clinical factors		*No of women with inclusion count*			
Ectopy	*OC*	*Low (<100 iwc)*	*High (>1000 iwc)*	*Total*	*% with high count*
−	−	50	23	73	32
−	+	26	24	50	48
+	−	52	54	106	51
+	+	68	106	174	61
Total		196	207	403	

+ Present − absent
iwc = inclusions per whole coverslip (10 mm diam)
OC = Oral contraceptives

Age factor

Of the total of 403 women with chlamydial infections, 114 (28%) were under 20 years of age (table II); of these, 76 (67%) had high inclusion counts compared with 131 (45%) of the 289 women over 20 years of age ($\chi_1^2 = 14\cdot9$, $P<0\cdot001$).

Despite this significant difference in the degree of infection between the under-20 age group and older women, no significant differences emerged in any of the other clinical features (table V).

Other clinical features

There were 119 women with one or more previous children and 22 pregnant women in the group examined; 36 women had associated trichomonal infections, 89 were infected with yeasts, and 31 had signs or symptoms suggesting salpingitis. There was no evidence that the frequency of high chlamydial counts was greater in these women than in women without these clinical features. However, the small numbers limit conclusive statistical analysis.

No significant difference was found in the proportion of women with high inclusion counts in successive weeks of the menstrual cycle. In the group of 403 women studied, 22 were pregnant, 32 had irregular periods or amenorrhoea, and in 20 the date of the last period was not recorded. In the 329 (82%) women with a regular cycle of 26-30 days, the month was divided into four weeks starting from the first day of menstruation, and the cycle-week in which the primary cervical swab for *C trachomatis* isolation was taken was recorded against the inclusion count obtained on culture (table VI).

Discussion

Current tissue culture methods for isolating *C trachomatis* from cervical swabs of infected women provide a quantitative means of monitoring the reproducibility and sensitivity of the procedures themselves in continued diagnostic use[2 4] and of detecting differences in the degree of infection between individual patients, since the number of inclusions which develop in McCoy cell culture is directly proportional to the number of viable chlamydial elementary bodies inoculated into the culture.[7]

TABLE V *Distribution of clinical features in relation to age*

Clinical feature	*% of women*	
	<20 years	*>20 years*
Mucopus	51	53
Gonorrhoea	40	39
Ectopy	75	68
Oral contraceptives	52	57

In the present series of 580 women with chlamydial genital infections from whom a primary cervical swab was taken for laboratory culture before they had been given chemotherapeutic agents, the inclusion counts were normally distributed with a median value of 450 iwc, but the range of counts obtained was wide, with 73 (13%) of the group having counts of >10 000 iwc whereas 84 (14%) had counts of <10 iwc. When the inclusion counts in the individual patients were related to the clinical features found at the time the swab was taken, the degree of infection (indicated by the inclusion count) apparently varied according to the presence or absence of cervical mucopus or ectopy, the use of oral contraceptives, and the age of the patient.

The increase in inclusion counts in women with cervical mucopus may be due to the following: (a) the greater the amount of secretion in the cervical canal the greater is the amount that might be collected on the swab, and hence the greater the number of elementary bodies available to infect the McCoy coverslip culture; (b) the presence of mucopus might non-specifically enhance the sensitivity of McCoy cells to chlamydial infection, resulting in a higher proportion of the potentially infective particles inoculated successfully forming inclusions in McCoy cells; (c) mucopus may be merely an indicator of another concurrent infection, especially gonorrhoea, which might be capable of enhancing the chlamydial infection—that is, *C trachomatis* might thus show the characteristics of a secondary opportunistic invader; and (d) mucopus might in fact be the direct result of chlamydial infection and thus occur most frequently in those with the greater degree of infection, as indicated by the inclusion count.

In our experience the thick tenacious nature of the mucopus often results in rather less than more cervical secretion being obtained per swab and limits the amount of homogenisation of material from the swab before tissue culture inoculation. Suspensions of cervical mucopus from patients with STDs but without current chlamydial infection are slightly but consistently inhibitory to the growth of laboratory strains of *C trachomatis* in McCoy cultures (Hobson and Lee, unpublished data).

TABLE VI *Inclusion counts in relation to menstrual cycle*

	*Week of cycle**				
	1	*2*	*3*	*4*	*Total*
No of patients	60	112	79	78	329
No with high count (>1000 iwc)†	31	60	40	42	173
% with high count	52	54	51	54	53

*At the time of primary cervical swab
†Inclusion count in primary cervical swab

Concurrent infection with trichomonads, yeasts, or *N gonorrhoeae* appeared to have no effect on the degree of chlamydial infection. It is interesting however that women with gonorrhoea have previously been found[8 9] to have a higher rate of chlamydial cervical infection than have contacts of men with NGU or attenders of STD clinics without gonorrhoea. This incidence may be increased because coincidental infections in the more promiscuous STD clinic patients, who must frequently be exposed to both types of infective agent, are highly likely rather than because gonorrhoea predisposes to or stimulates a previously latent or low-grade chlamydial infection. Furthermore, women who had concomitant infections did not develop mucopus more frequently than did women with chlamydial infections alone; in both groups the presence of mucopus was associated with high chlamydial inclusion counts. These results thus suggest that the presence of mucopus in a woman with gonorrhoea may, in certain cases, be the result of an associated chlamydial infection rather than due to the gonococcal infection alone.

There is a highly significant association between the chlamydial isolation rate and the incidence of cervicitis in contacts of men with NGU,[3 10] and treatment of the chlamydial infection is quickly followed by disappearance of mucopus.[3] The present results support and extend these findings—that is, *C trachomatis* can cause endocervicitis and the greater the degree of infection the more likely are the signs of endocervicitis to be present.

In contacts of men with NGU seen in Liverpool[3] a higher rate of chlamydial infection was found when both cervical ectopy and contraceptive steroids were present than when either occurred alone or when neither was present. All the infected women from this smaller study group[3] are included in the present larger and more varied series, which shows that not only the incidence but also the degree of chlamydial infection was greater in women with ectopy and contraceptive steroids than in women with neither. Oral contraceptives alone did not appear to have any influence on the degree of infection but, in contrast, the inclusion counts were significantly higher in those with ectopy alone—even in the absence of oral contraceptives—than in women without ectopy. Since the site of the squamo-epithelial junction is determined during fetal life,[11] infection with *C trachomatis* cannot be a cause of cervical ectopy, although infection may convert a small area of simple ectopy to a larger area of hypertrophic ectopy as a result of oedema and congestion.[3]

The most likely explanation of the significant association between the presence of ectopy and both an increased rate of infection[3] and an increase in the degree of that infection demonstrable in tissue culture is simply that the greater the area of columnar epithelium exposed on the vaginal aspect of the cervix the greater is the chance that infection will be acquired after coitus with an infected man, the greater is the number of susceptible columnar cells available for progressive growth cycles of the infective agent, and the greater is the area that can be sampled by taking swabs.

The role of oral contraceptives is more difficult to determine and is almost certainly multifactorial. The frequency and size of cervical ectopy is itself increased by the use of contraceptive steroids,[12 13] and thus the chlamydial inclusion count is likely to be significantly increased if oral contraceptives are considered overall, but much of this increase may be the result of the ectopy associated with the use of oral contraceptives and not uniquely due to the drugs. However, oral contraceptives may possibly also have direct hormonal effects on columnar epithelium in vivo which increases the chance that a highly productive infection with *C trachomatis* will develop. Contraceptive steroids have a progestational effect, producing a large outflow of alkaline mucus from the endocervical glands.[12] The addition of steroids to McCoy cell cultures increases their susceptibility to infection with laboratory strains of *C trachomatis,* resulting in an increase in the number of infective progeny produced by the inoculation of a given number of elementary bodies.[14] Thus, as suggested by the present findings, the hormonal effects of oral contraceptives would be seen most clearly when acting in conjunction with cervical ectopy, especially since the action of these steroids increases the bulk of the cervix and produces eversion.[12] However, in the present series of women there was no significant association between the height of the inclusion count and other hormonal influences, either in the normal menstrual cycle or in pregnancy, although a much larger series of women would need to be examined to confirm the latter.

A further feature needing more extensive investigation is the highly significant association between the age of the patient and the degree of her infection in the present series of women. This association might possibly reflect anatomical and physiological, including hormonal, differences between women under 20 years of age and older women, but the present findings do not suggest that the increased inclusion counts are the results of a greater use of oral contraceptives or of a greater frequency of cervical ectopy than in women between 21-45 years of age. However, the exact area of ectopy was not measured in these women. The size of cervical ectopy diminishes with age owing to metaplasia, whereby columnar epithelium is gradually replaced by squamous epithelium, and this process is often initiated at puberty or in the first pregnancy.[11] Thus, possibly the area of ectopic columnar cells, rather than the frequency of ectopy, may vary between young and older women to influence their differing degrees of chlamydial infection.

It is also possible that this age difference may have an immunological basis. In girls aged between 14-20 years a greater proportion of the infections are probably the first chlamydial infections to which they have been exposed than is the case in older women with a longer period of sexual activity. Thus, the replication of *C trachomatis* may be less likely to be brought quickly under control by humoral or cervical antibody or by cell-mediated immune responses in young women than in older women with a greater chance of previous immunological experience. Humoral antibody against *C trachomatis* is frequent and persistent in women attending STD clinics and its frequency increases with age.[15-17] Although it is not yet clear that antibody acquired by previous experience will prevent infection, the possibility that it might modify the degree of infection and hence the clinical signs associated with high-inclusion count infection merits further study.

This work was supported by a project grant from the Medical Research Council.

References

1. Rees E, Tait IA, Hobson D, Johnson FWA. Chlamydia in relation to cervical infection and pelvic inflammatory disease. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:67-76.
2. Johnson FWA, Hobson D, Rees E, Tait IA. Quantitative aspects of the growth of *Chlamydia trachomatis* in diagnostic tissue culture procedures. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:309-13.
3. Tait IA, Rees E, Hobson D, Byng RE, Tweedie MCK. Chlamydial infection of the cervix in contacts of men with nongonococcal urethritis. *Br J Vener Dis* 1980;**56**:37-45.
4. Johnson FWA, Hobson D. Factors affecting the sensitivity of replicating McCoy cells in the isolation and growth of *Chlamydia* A (TRIC agents). *J Hyg Camb* 1976;**76**:441-51.
5. Davies JA, Rees E, Hobson D, Karayiannis P. Isolation of *Chlamydia trachomatis* from Bartholin's ducts. *Br J Vener Dis* 1978;**54**:409-13.
6. Johnson FWA, Chancerelle LYJ, Hobson D. An improved method for demonstrating the growth of chlamydiae in tissue culture. *Med Lab Sci* 1978;**35**:67-74.
7. Gordon FB, Dressler HR, Quan AL, Thomas JJ. Effect of ionizing irradiation on susceptibility of McCoy cell cultures to *Chlamydia trachomatis. Appl Microbiol* 1972;**23**:123-9.
8. Hobson D, Rees E. The role of *Chlamydia trachomatis* in female genital infections and in conjunctivitis of the newborn. In: Brumfitt W, ed. *New Perspectives in Clinical Microbiology.* London: Kluwer Medical, 1978:109-23.
9. Hilton AL, Richmond SJ, Milne JD, Hindley F, Clarke SKR. *Chlamydia* A in the female genital tract. *Br J Vener Dis* 1974;**50**:1-10.
10. Dunlop EMC, Al-Husseini MK, Freedman A, *et al.* Infection by TRIC agent and other members of the Bedsonia group; genital infection and disease of the eye. *Trans Ophthalmol Soc UK* 1966;**86**:321-34.
11. Singer A, Jordan JA. The anatomy of the cervix. In: Jordan JA, Singer A, eds. *The Cervix.* London: WB Saunders, 1976:13-36.
12. Jordan JA, Singer A. Effect of oral contraceptive steroids upon epithelium and mucus. In: Jordan JA, Singer A, eds. *The Cervix.* London: WB Saunders, 1976:192-207.
13. Goldacre MJ, Loudon N, Watt B, *et al.* Epidemiology and clinical significance of cervical erosion in women attending a family planning clinic. *Br Med J* 1978;**1**:748-50.
14. Bushell AC, Hobson D. Effect of cortisol on the growth of *C trachomatis* in McCoy cells. *Infect Immun* 1978;**71**:946-53.
15. Richmond SJ, Caul EO. Single-antigen indirect immunofluorescence test for screening venereal disease clinic populations for chlamydial antibodies. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:259-65.
16. Treharne JD, Dines RJ, Darougar S. Serological responses to chlamydial ocular and genital infections in the United Kingdom and Middle East. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:249-58.
17. Schachter J, Dawson CR. Comparative efficiency of various diagnostic methods for chlamydial infection. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:336-41.

Br J Vener Dis 1980;56:163-8.

Epidemiology of infection by serotypes D to K of Chlamydia trachomatis*

ERIC M C DUNLOP,† SOHRAB DAROUGAR,‡ AND JOHN D TREHARNE‡
From the †Whitechapel Clinic, The London Hospital, and the ‡Virus Laboratory, WHO Collaborating Centre for Reference and Research on Trachoma and other Chlamydial Infections, Department of Clinical Ophthalmology, Institute of Ophthalmology, University of London, London

SUMMARY Non-specific urethritis (NSU) is a sexually transmitted disease; 50% of cases are due to *Chlamydia trachomatis,* so that this is the commonest sexually transmitted infection in the developed world. Chlamydial infection is now readily diagnosable and the evidence increasingly suggests that it is underdiagnosed. Chlamydial conjunctivitis (in the newborn baby or the adult) in the developed world is a complication of sexually transmitted genital infection by *C trachomatis* and it indicates a large reservoir of such infections. Because of the association of sexually transmitted diseases, systemic treatment for such chlamydial conjunctivitis should not be given until full genital and serological investigations have been carried out. Chlamydial infection causes serious complications (that were formerly often thought to be gonococcal), such as epididymitis in young men and salpingitis in young women. It may cause local complications in the eye of the newborn baby and even pneumonia in babies and fatal endocarditis in adults.

The diagnosis of NSU should lead to the correct treatment of the male patient and of his sexual partners. It is the promiscuous woman, who does not have a regular sexual partner to report back to her that he has NSU, who is at particular risk of undiagnosed chlamydial infection. Routine genital investigations for chlamydia are particularly indicated in her case.

Following the parallel of gonorrhoea, it seems that the use of contact tracers may be an effective method for controlling chlamydial infection.

Introduction

Urethritis is gonococcal or nongonococcal. Nongonococcal urethritis (NGU) is due to many specific causes identifiable by history and simple investigations; these include the sexually transmitted infestations and infections due to *Trichomonas vaginalis* and herpes genitalis as well as agents which are not sexually transmitted—for example, urinary tract infections causing secondary bacterial urethritis, chemicals, and trauma by instruments, catheters, and foreign bodies. Exclusion of these causes by history and simple investigations leaves what was called non-specific or abacterial urethritis (NSU), for which no specific cause could be ascribed. The development of efficient investigations for chlamydia (particularly cell culture) has shown that about 50% of cases of NSU are due to the D to K serotypes of *Chlamydia trachomatis.*[1-6] In patients with frank discharge, the isolation rate may reach 75%.[6]

NON-SPECIFIC URETHRITIS

It will be many years before small clinics have the facilities for investigations for chlamydia. Until they do the diagnosis of NSU (always a sexually transmitted infection) is a useful pragmatic one that should lead to correct treatment and management of the patient and sexual contacts. Both chlamydial and non-chlamydial NSU respond to tetracycline.

NONGONOCOCCAL URETHRITIS

NGU was first reported in England and Wales in 1951 when there were 10 794 cases in men; by 1974, these had increased to 72 425 in men alone compared with 59 754 cases of gonorrhoea in men, women, and children. Allowing for the female partners of the men with NGU and for babies with chlamydial ophthalmia, total cases must have numbered over 140 000 in the year. Thus NGU is the commonest disease

---

*Paper presented in part at the symposium on sexually transmitted disease of the British Society for the Study of Infection, 3 November 1978

Address for reprints: Dr E M C Dunlop, The Whitechapel Clinic, The London Hospital, London E1 1BB

Received for publication 3 September 1979

complex reported from clinics in England and Wales. Because over 90% of NGU is NSU (and about half of that is chlamydial) it seems that chlamydial infection is the commonest sexually transmitted infection in England and Wales, the United States,[7 8] and probably in the developed world.

In 1951 at the Whitechapel Clinic, 751 cases of NGU were diagnosed; in 1976 (partly as a result of improvements in diagnosis) this number had increased to 2333 (an increase of 196%), of which 2161 (97%) cases were due to NSU.

OPHTHALMIA NEONATORUM

Infection of the conjunctiva in the developed world may be likened to the tip of an iceberg indicating a massive reservoir of genital infection. Thus, Neisser in 1897[9] originally described *Neisseria gonorrhoeae* in material from the eyes of newborn babies and the genital tracts of adults. Soon ophthalmia neonatorum was recognised as gonococcal or nongonococcal, the latter being due to bacteria other than *N gonorrhoeae* or abacterial. Over 90 years ago, Kroner in 1884[10] thought that the nongonococcal forms were due to infection from the genital tract of the mother. After Halberstaedter and von Prowazek in 1907[11] described the inclusions now known by their names, these inclusions were found in conjunctival material from newborn babies and adults with conjunctivitis and in genital material from adults. After 50 years had elapsed, culture in yolk sac by T'ang and others in 1957[12] first permitted isolation of *C trachomatis* from infected sites. Culture in cell culture[13] is more effective than in yolk sac;[14] it has been modified and made even simpler and more sensitive. Thus we can now readily isolate the agent from affected babies, from over 90% of their mothers,[15 16] from the fathers with NSU, from about 50% of men attending clinics with NSU (as already noted), and from the eyes of young adults who present with chlamydial conjunctivitis and from their genital tracts.[17]

Chlamydial infection in pregnancy

Prospective studies of pregnant women in the United States of America have shown that about 5-13% may yield chlamydia-positive cervical secretions; about half of the babies born to culture-positive women develop clinical conjunctivitis but more are infected, as shown by the presence of antichlamydial antibody in tears or serum.[18-20] Chlamydial pneumonia may develop even without previous clinical conjunctivitis.[21-23] Schachter and others[24] reported an estimated incidence of chlamydial infection of 28/1000 live births with 14 cases of conjunctivitis and eight of pneumonia in a study in San Francisco.

Incidence of chlamydial and gonococcal ophthalmia

Chlamydial ophthalmia neonatorum (formerly called inclusion conjunctivitis or inclusion blenorrhoea) is much commoner than gonococcal ophthalmia neonatorum.[16] In the time that 34 babies with chlamydial ophthalmia were seen at the London Hospital and Institute of Ophthalmology, only five were seen because of gonococcal ophthalmia neonatorum; a previous study reported this ratio as 44 : 8.[25] Similarly, in the time that 153 adults were seen because of fresh chlamydial infection of the eye, only one adult with gonococcal infection of the eye was seen. The difference reflects the higher prevalence of chlamydial infection of the genitalia and the fact that chlamydial urethritis is often less productive of symptoms than gonococcal urethritis, so that it more often remains undetected. Thus investigation of the overnight urethral secretion may be necessary to make the diagnosis of NSU or chlamydial urethritis.[26 27]

COMPLICATIONS OF CHLAMYDIAL INFECTION

Chlamydial ophthalmia neonatorum is more serious than formerly thought. It is a local manifestation of a more generalised infection in the baby.[28] It indicates potentially serious infection in the mother with certainty and in the father with strong probability. Pelvic inflammation is common in infected mothers after delivery.[15 16 29 30] Isolation of *C trachomatis* from infected Fallopian tubes has confirmed that this agent is a major cause of salpingitis;[31-33] isolation from Bartholin's ducts shows that it causes bartholinitis.[34] It is the commonest cause of epididymitis in young men.[35] Because *C trachomatis* is sexually transmitted, it is commonly associated with other sexually transmitted infections;[3 15 16 29 34 36 37] thus ophthalmia neonatorum may be due to gonococci and chlamydia together,[16] just as may genital infection in adults.

Chlamydial ophthalmia neonatorum may cause local complications in the eye if treatment is started after the thirteenth day of life,[30] or if reinfection occurs.[38]

The incubation of chlamydial ophthalmia is often longer than that of the gonococcal form, but chlamydial ophthalmia may be present at birth and both forms may occur after caesarian section, if this is carried out after the membranes have ruptured. Infants are commonly premature,[16] which may be due to infection of fetal membranes over the infected cervix.

In one series of 25 mothers of infected babies,[15] only 19 of the fathers were examined. As with gonococcal ophthalmia there is sexual instability in the parents of the affected babies; not all of the "fathers" were the natural fathers of the babies; they were the current sexual partners of the mothers.

TABLE I *Development of chlamydial urethritis —Mr H, a student aged 24 years, was father of a baby with chlamydial ophthalmia neonatorum; his wife* was chlamydia-positive*

Age of baby (weeks)	*LMI (days)*	*Symptoms*	*Urethritis*	*Urethral PMNL/HPF*	*Hours urine held*	*Chlamydial culture result*
5	5	None	No	3	7	–
12	21	None	No	2	5½	–
13	1	Urethral discharge (1 day)	Yes	10	2	+
14	8	Urethral discharge (8 days)	Yes	100	9	+

*Mrs H had a positive cervical culture result for chlamydia after one week's treatment with oxytetracycline 250 mg four times daily and a negative result after 500 mg four times daily for two weeks
+ Positive – negative
LMI = last marital intercourse
PMNL = polymorphonuclear leucocytes
HPF = high power field (× 1000)

Table I shows the development of urethritis in one father (Mr H*) after resumption of marital intercourse when the baby was just over 4 weeks old. It seems that this man infected his wife before or during her pregnancy. His urethritis had cleared, to recur when his baby was just under 13 weeks old. *C trachomatis* could only be isolated when he had urethritis. After one week of treatment with oxytetracycline 250 mg four times a day by mouth (after milkproduct-free meals), *C trachomatis* was again isolated from his wife from cervical material. Since then a standard treatment of oxytetracycline 500 mg, four times a day for 14 days, has been used.

It is now becoming clear that *C trachomatis* may cause serious complications, some of which resemble infection by *Chlamydia psittaci.* Thus, apart from infection of sexual partners and the eyes of newborn babies, spread of infection from genitalia to the eye, salpingitis, and epididymitis, *C trachomatis* has been shown to cause endocarditis that was only diagnosed after the death of an adult with a previously normal heart,[39] and to cause pneumonia in babies.[21-23]

EXPERIMENTAL CHLAMYDIAL URETHRITIS

Chlamydia-positive urethritis has been produced in male baboons by inoculation with cultures of D to K serotypes of *C trachomatis* by different workers.[40,41] The latter authors produced urethritis that remained chlamydia-positive for three months; of four attempts at reinfection, three were successful; in each case urethritis remained isolate-positive for less than 15 days. Reinfection is obviously possible in subhuman primates (and man) but partial immunity may develop.

MANAGEMENT OF GENITAL INFECTIONS

Some recurrences of NSU are due to inadequate treatment and follow up. Others are due to defective management so that infected sexual partners are not treated. Thus, Mr NT, a medical worker aged 26, was seen because of chlamydia-positive urethritis, only nine days after he had been told he was "cured" of NSU after a five-day course of tetracycline 500 mg twice daily at another hospital. He denied having had sexual intercourse (or contact) since this treatment. This patient had had no less than four previous attacks of NSU in the two years before attendance. On only one of these occasions had his then regular girlfriend been treated; that was with a similar short course of tetracycline and was not concurrent with his treatment, so that "ping-pong" reinfection may have occurred. After treatment with doxycycline 200 mg daily for 14 days smears of the overnight urethral secretion on three occasions with cultures for chlamydia gave negative results.

The female sexual partners of men with chlamydial urethritis commonly have chlamydial infection of the cervix and the urethra.[1,2,42] Chlamydia may also be isolated from the rectum of such women[43] and, rarely, from the throat,[44,45] as well as from the urethra, the rectum, and the throat of male homosexuals.[46] Contact tracing for NSU usually results in the attendance of subsequent, rather than source, contacts, but Oriel and others[47] isolated chlamydia from eight of 13 source contacts and from six of 24 subsequent contacts.

HYPERENDEMIC TRACHOMA (SEROTYPES A-C)

In the developing countries hyperendemic trachoma due to serotypes A to C of *C trachomatis,* which is the commonest eye disease in the world, may affect up to 90% of children in areas of high prevalence by the age of 1 year. The disease is spread by eye-to-eye transmission ("ocular promiscuity"), by flies and poor hygienic practices.[48] Spread is helped by the lack of piped water for washing and the persistence of infectious discharges from the eye. Some 500 million people are affected and some two million are blinded.[49] The disease is caused by infection and superinfection by the A, B, Ba, and C serotypes of *C trachomatis,* together with superadded bacterial infections.

---

*The initials used in the case histories are those of a coding system.

OCULAR INFECTION (SEROTYPES D-K)

In the developed countries, the D to K serotypes of *C trachomatis* infect the genital tract and cause chlamydial infection of the eye because of contamination with infected genital secretion. Such material was probably the cause of most cases of "swimming bath conjunctivitis." Only rarely will a sibling of an infected baby, or an adult, develop chlamydial infection of the eye because of contamination of the eye with infected ocular material.

In a series of 23 adult women who had presented because of isolate-positive ocular infection, *C trachomatis* was isolated from the genital tract in 19 cases; from the cervix in 19 of 21, the urethra in seven of 13, and the rectum in 10 of 22.[17] One of these patients (Mrs OD; table II) had had chlamydial conjunctivitis for eight months and had probably harboured the organism for three years in genitalia and rectum, where she had the most marked mucosal changes.[43] Certainly genital infection may persist for prolonged periods. Schachter and others[3] have reported persistence of chlamydial infection of the genitalia for one year in a man and for 11 months in a woman. Rees and others[50] reported persistence of chlamydial infection for up to 19 weeks in 18 women and for one year in another.

TABLE II *Case histories of a married couple with chlamydial infections for over three years*

MRS OD, a model, married for 4 months, seen on 14 April 1970.
History—sore left eye for 8 months, treated with Albucid and referred to Moorfields Eye Hospital; diagnosis, TRIC punctate kerato-conjunctivitis (TPK). Last marital intercourse occurred 1 day before; premarital intercourse for 3 years; husband only partner.
Cervix showed "follicles" and pus cells (60/HPF); rectum showed giant "follicles" and pus cells (20/HPF).
Culture—eye, cervical, and rectal cultures chlamydia-positive.

MR OD, seen on 21 April 1970.
History—last marital intercourse 8 days before; premarital intercourse for 3 years; no other contact for more than 3 years.
Symptoms—occasional slight dysuria.
Signs—slight urethritis. Microscopy—first-voided urine: shreds (14 PMNL/HPF); second urine: clear; urine held for 14 hours.
Culture—Meatal swab and urethral scrape chlamydia-positive.

PMNL = polymorphonuclear leucocytes; HPF = high-power field

CONTACT TRACING

Of the male sexual partners of the 23 women reported by Vaughan-Jackson and others,[17] 10 were examined. *C trachomatis* was isolated from urethral material from five (including Mr OD; table II), all of whom had urethritis.

TABLE III *Case histories of three patients with chlamydial infections associated with gonorrhoea*

MRS TI, aged 22, a computer operator and barmaid from Eastbourne.
History—November 1976: conjunctivitis diagnosed and treated with cephalexin tablets. 8 February 1977: chlamydia-positive conjunctivitis diagnosed and treated with doxycycline 300 mg immediately and 100 mg daily for 21 days; same treatment given to husband (Mr TI). 12 July 1977: chlamydia-positive conjunctivitis diagnosed and treated locally. 3 August 1977: genital investigations performed; chlamydia-positive cervicitis and urethritis diagnosed; cervical and urethral cultures positive for *N gonorrhoeae.*

MR TI, aged 23, a lift repairer from Eastbourne.
History—February 1977: treated with doxycycline 100 mg daily for 21 days.
Symptoms—none; on examination, urethritis after holding urine for 12 hours.
Microscopy—Gram stains showed 200 PMNL/HPF.
Culture—negative culture result for *N gonorrhoeae,* positive result for *C trachomatis.*

MR JT, aged 25, an asphalter from Eastbourne.
Symptoms—urethral discharge for 6 weeks, bilateral testicular pain for 1 week. On examination, bilateral epididymitis.
Culture—positive results for *N gonorrhoeae* and for *C trachomatis* (developed postgonococcal urethritis).

MRS IH, divorced, aged 22, housewife from Eastbourne.
Symptoms—abdominal pain for two weeks. Appendicectomy performed, bilateral salpingitis found; treated with ampicillin and penicillin intramuscularly for 7 days.
Culture—negative results for *N gonorrhoeae* and *C trachomatis.*
Serology—LGVCFT positive (1/64); micro-IF test positive; $\geqslant$256 A-K.

Control of a sexually transmitted disease must include control of infection in the sexual partners.

The case of Mrs TI (table III), in which conjunctivitis recurred until suitable contact tracing had been carried out, illustrates this point and the association of chlamydial infection with other sexually transmitted infections. Examination showed chlamydial infection of the genitalia which had recurred despite her having been given doxycycline for herself and her husband (Mr TI) (without previous genital examination). It also showed that she had gonorrhoea, which was found in her extramarital consort (Mr JT), who had been virtually symptomfree until he had developed epididymitis. Chlamydial urethritis was present in her husband. Her extramarital consort had both chlamydial and gonococcal urethritis with epididymitis. One of his contacts (Mrs IH) had developed acute abdominal pain, diagnosed as due to appendicitis. Operation showed that she had acute bilateral salpingitis that was virtually certainly chlamydial with or without gonococcal infection.

Male contacts of women with chlamydial conjunctivitis included Mr VZ, aged 21, the regular sexual partner for three years of a woman with chlamydial conjunctivitis and genital infection. He had had a slight urethral discharge for two months, which was found to be chlamydial. Five months earlier he had been successfully treated for NSU elsewhere; but neither his regular sexual partner nor his other recent contact had been seen so that "ping-pong" reinfection resulted and his regular sexual partner developed chlamydial infection of the eye.

Of 21 men with chlamydial infection of the eye,[17] chlamydial urethritis was present in eight. An additional four who had no evidence of urethritis and had negative genital culture results for chlamydia had female sexual partners who harboured chlamydia in the genital tract; this suggests that immunity of the genital tract may develop and may not parallel that of the eye.

The incidence of chlamydial infection of the genitalia in women attending clinics in the United Kingdom varies from 12% to 31%.[37 47 51-54] It seems no coincidence that the highest incidence was found in a clinic[54] in which the contacts of men with NSU were not routinely seen and treated.

The use of contact tracers at the Whitechapel Clinic of The London Hospital led to a 20% decrease in the incidence of gonorrhoea at a time when the national incidence had increased by 33%. The decrease was in the number of infected men attending because the number of infected women attending had increased. Thus the ratio of infected women to men was 1 : 3·9 in 1960 and 1 : 2·2 in 1969.[55]

Following the parallel of gonorrhoea, it is likely that the use of contact tracers may provide an effective way of improving the control of chlamydial infection.

Conclusions

NSU is a sexually transmitted disease and about 50% of cases are due to *C trachomatis*. A diagnosis of NSU in men does not require complicated investigations and should lead to the examination of the sexual partner(s), the exclusion of other sexually transmitted diseases, and effective treatment with tetracycline, which is effective against chlamydial and non-chlamydial NSU.

The promiscuous woman, who does not have a regular sexual partner to report to her that he has urethritis, is at special risk of having undetected chlamydial infection of the genitalia. Routine genital investigations for chlamydia are particularly indicated in her case.

References

1. Dunlop EMC, Vaughan-Jackson JD, Darougar S, Jones BR. Chlamydial infection: incidence in "non-specific" urethritis. *Br J Vener Dis* 1972;**48**:425-8.
2. Holmes KK, Handsfield HH, Wang S-P, *et al.* Etiology of non-gonococcal urethritis. *N Engl J Med* 1975;**292**:1199-205.
3. Schachter J, Hanna L, Hill EC, *et al.* Are chlamydial infections the most prevalent venereal disease? *JAMA* 1975;**231**:1252-5.
4. Oriel JD, Reeve P, Wright JT, Owen J. Chlamydial infection of the male urethra. *Br J Vener Dis* 1976;**52**:46-51.
5. Perroud HM, Miedzybrodzka K. Chlamydial infection of the urethra in men. *Br J Vener Dis* 1978;**54**:45-9.
6. Terho P. *Chlamydia trachomatis* in non-specific urethritis. *Br J Vener Dis* 1978;54:251-6.
7. Gale J, Hinds W. Discussion. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:47.
8. Wiesner PJ. Selected aspects of the epidemiology of nongonococcal urethritis. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:9.
9. Neisser A. Uebe eine der gonorrhoea eigentumliche micrococcusform. *ZBl Med Wiss* 1879;**17**:497-500.
10. Kroner T. Zur äetiologie der ophthalmablennorrhoea neonatorum. *ZBl Gynäk* 1884;**8**:643-5.
11. Halberstaedter L, Prowazek S von. Zur äetiologie des trachoms. *Dtsch Med Wochenschr* 1907;**2**:1285-7.
12. T'ang F-F, Chang H-L, Huang Y-T, Wang K-C. Studies on the etiology of trachoma with special reference to isolation of the virus in chick embryo. *Chin Med J* 1957;**75**:429-47.
13. Gordon FB, Quan AL. Isolation of the trachoma agent in cell culture *Proc Soc Exp Biol Med* 1965;**118**:354-9.
14. Gordon FB, Harper IA, Quan AL, Treharne JD, Dwyer RStC, Garland JA. Detection of *Chlamydia* (Bedsonia) in certain infections of man. 1. Laboratory procedures: comparison of yolk sac and cell culture for detection and isolation. *J Infect Dis* 1969;**120**:451-62.
15. Dunlop EMC, Goldmeier D, Darougar S, Jones BR. Chlamydial infection of the genital tract in mothers and fathers of babies suffering from ophthalmia neonatorum due to TRIC agent. In: Catterall RD, Nicol CS, eds. *Sexually Transmitted Diseases.* London, New York, San Francisco: Academic Press, 1976:83-8.
16. Rees E, Tait IA, Hobson D, Byng RE, Johnson FWA. Neonatal conjunctivitis caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis. Br J Vener Dis* 1977;**53**:173-9.
17. Vaughan-Jackson JD, Dunlop EMC, Darougar S, Dwyer RStC, Jones BR. Chlamydial infection: Results of tests for *Chlamydia* of patients suffering from acute Reiter's disease compared with results of tests of genital tract and rectum of patients with ocular infection due to TRIC agent. *Br J Vener Dis* 1972;**48**:445-51.
18. Alexander ER, Chandler J, Pheiffer TA, Wang S-P, English M, Holmes KK. Prospective study of perinatal *Chlamydia trachomatis* infection. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977; 148-52.
19. Chandler JW, Alexander ER, Pheiffer TA, Wang S-P, Holmes KK, English M. Ophthalmia neonatorum associated with maternal chlamydial infection. *Trans Am Acad Ophthalmol Otolaryngol* 1977;**83**(2):302-8.
20. Schachter J, Dawson CR. *Human Chlamydial Infections.* Littleton, Mass: PSG Publishing Company Inc, 1978:117-20.
21. Beem MO, Saxon EM. Respiratory tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis. N Engl J Med* 1977;**296**:306-10.
22. Beem MO, Saxon EM. Distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis.* In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:153-5.
23. Hammerschlag MR. Chlamydial pneumonia in infants. *N Engl J Med* 1978;**298**:1083-4.
24. Schachter J, Grossman M, Holt J, Sweet R, Goodner E, Mills J. quoted by Schachter J, Dawson CR. Is trachoma an ocular component of a more generalised chlamydial infection? *Lancet* 1979;**1**:702-3.
25. Dunlop EMC, Jones BR, Darougar S, Treharne JD. Chlamydia and non-specific urethritis. *Br Med J* 1972;**2**:575-7.
26. Rodin P. Asymptomatic non-specific urethritis. *Br J Vener Dis* 1971;**47**:452-3.
27. Simmons PD. Evaluation of the early morning smear investigation. *Br J Vener Dis* 1978;**54**:128-9.
28. Schachter J, Dawson CR. Is trachoma an ocular component of

a more generalised chlamydial infection? *Lancet* 1979;**1**:702-3.

29. Dunlop EMC, Freedman A, Garland JA, *et al.* Infection by Bedsoniae and the possibility of spurious isolation. 2. Genital infection, disease of the eye, Reiter's disease. *Am J Ophthalmol* 1967;**63**:1073-81.
30. Mordhorst CH, Dawson C. Sequelae of neonatal inclusion conjunctivitis and associated disease in parents. *Am J Ophthalmol* 1971;**71**:861-7.
31. Eilard T, Brorsson J-E, Hamark B, Forssman L. Isolation of *Chlamydia* in acute salpingitis. *Scand J Infect Dis* 1976;suppl **9**:82-4.
32. Hamark B, Brorsson J-E, Eilard T, Forssman L. Preliminary report: salpingitis and chlamydiae subgroup A. *Acta Obstet Gynecol Scand* 1976;**55**:377-8.
33. Mårdh P-A, Ripa T, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977;**296**:1377-9.
34. Davies JA, Rees A, Hobson D, Karayiannis P. Isolation of *Chlamydia trachomatis* from Bartholin's ducts. *Br J Vener Dis* 1978;**54**:409-13.
35. Berger RE, Alexander ER, Morida GD, Ansell J, McCormick G, Holmes KK. *Chlamydia trachomatis* as a cause of acute idiopathic epididymitis. *N Engl J Med* 1978;**298**:301-4.
36. Ridgway GL, Oriel JD. Inter-relationship of *Chlamydia trachomatis* and other pathogens in the female genital tract. *J Clin Pathol* 1977;**30**:933-6.
37. Woolfitt JMG, Watt L. Chlamydial infection of the urogenital tract in promiscuous and non-promiscuous women. *Br J Vener Dis* 1977;**53**:93-5.
38. Mordhorst CH, Grayston JT, Wang S-P. Childhood trachoma in a nonendemic area. Danish trachoma patients and their close contacts 1963-1973. *JAMA* 1978;**239**:1765-71.
39. Van der Bel-Kahn JM, Watanakunakorn C, Menefee MG, Long HD, Dicter R. *Chlamydia trachomatis* endocarditis. *Am Heart J* 1978;**95**:627-36.
40. Darougar S, Kinnison JR, Jones BR. Chlamydial isolates from the rectum in association with chlamydial infection of the eye or genital tract. 1. Laboratory aspects. In: Nichols RL, ed. *Trachoma and Related Disorders*. Amsterdam, London, Princeton: Excerpta Medica, 1971:501-6.
41. Digiacomo RF, Gale JL, Wang S-P, Kiviat MD. Chlamydial infection of the male baboon urethra. *Br J Vener Dis* 1975;**51**:310-3.
42. Oriel JD, Reeve P, Powis A, Miller A, Nicol CS. Chlamydial infection: isolation of *Chlamydia* from patients with non-specific genital infection. *Br J Vener Dis* 1972;**48**:429-36.
43. Dunlop EMC, Hare MJ, Darougar S, Jones BR. Chlamydial isolates from the rectum in association with chlamydial infection of the eye or genital tract. II Clinical aspects. In: Nichols RL, ed. *Trachoma and Related Disorders*. Amsterdam, London, Princeton: Excerpta Medica, 1971:507-12.
44. Schachter J, Attwood G. Chlamydial pharyngitis. *J Am Vener Dis Assoc* 1975;**2**:12.
45. Bowie WR, Alexander ER, Holmes KK. Chlamydial pharyngitis. *J Am Vener Dis Assoc* 1978;**4**:140-1.
46. Goldmeier D, Darougar S. Isolation of *Chlamydia trachomatis* from throat and rectum of homosexual men. *Br J Vener Dis* 1977;**53**:184-5.
47. Oriel JD, Powis PA, Reeve P, Miller A, Nicol CS. Chlamydial infections of the cervix. *Br J Vener Dis* 1974;**50**:11-6.
48. Jones BR. The prevention of blindness from trachoma. *Trans Ophthalmol Soc Uk* 1975;**95**:16-33.
49. *Lancet*. Editorial: chlamydial infections of the eye. *Lancet* 1977;ii:857.
50. Rees E, Tait IA, Hobson D, Johnson FWA. *Chlamydia* in relation to cervical infection and pelvic inflammatory disease. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977:140-7.
51. Burns DC MacD, Darougar S, Thin RN, Lothian L, Nicol CS. Isolation of *Chlamydia* from women attending a clinic for sexually transmitted diseases. *Br J Vener Dis* 1975;**51**:314-8.
52. Hobson D, Johnson FWA, Rees E, Tait IA. Simplified method for diagnosis of genital and ocular infections with *Chlamydia*. *Lancet* 1974;ii:555-6.
53. Nayyar KC, O'Niell JJ, Hambling MH, Waugh MA. Isolation of *Chlamydia trachomatis* from women attending a clinic for sexually transmitted diseases. *Br J Vener Dis* 1976;**52**:396-8.
54. Hilton AL, Richmond SJ, Milne JD, Hindley F, Clarke SKR. *Chlamydia* A in the female genital tract. *Br J Vener Dis* 1974;**50**:1-10.
55. Dunlop EMC, Lamb AM, King DM. Improved tracing of contacts of heterosexual men with gonorrhoea. *Br J Vener Dis* 1971;**47**:192-4.

Br J Vener Dis 1980;**56**:169-72.

Ureaplasma urealyticum in the urethra of healthy men

J VIARENGO,† F HEBRANT,* AND P PIOT†

*From the *Unit of Biometry and the †Bacteriology Laboratory, Institute of Tropical Medicine, Antwerp, Belgium*

SUMMARY Nineteen healthy married white men had samples of first-voided urine cultured for *Ureaplasma urealyticum* at weekly intervals for 17 consecutive weeks. Of these, 63% harboured *U urealyticum* in their urine on at least one occasion; in 40% of the men with positive culture results ureaplasmas were isolated from 90% of samples. Those men whose specimens were frequently culture-positive had notably higher titres for *U urealyticum* than those whose specimens were less frequently positive. There was no significant correlation between the number of leucocytes in the urinary sediment and the amount of *U urealyticum* in the corresponding urine specimens, although ureaplasma-positive urine specimens tended to contain slightly more leucocytes than ureaplasma-negative samples. Fourfold or higher rises in titre of *U urealyticum* in urine samples was not associated with urethral signs or symptoms. One man with a presumably primary infection developed dysuria. Sixty-five strains of *U urealyticum* were serotyped, with types 2 and 3 predominating; the serotype found in each patient was fairly constant.

Introduction

The most convincing evidence that *Ureaplasma urealyticum* is associated with nongonococcal urethritis (NGU) has been given by Bowie and co-workers.[1] Among men experiencing a first episode of urethritis *U urealyticum* was recovered more often and in greater numbers from chlamydia-negative cases of NGU than from chlamydia-positive cases or from men without urethritis. Further support for a pathogenic role for *U urealyticum* has come from treatment studies[2-4] and from intraurethral inoculation of the organism into man.[5 6]

Nevertheless the question why *U urealyticum* is found in the urethra of a notable proportion of men without urethritis still remains unanswered; although the above studies suggest that *U urealyticum* is a pathogen in the urethra, they do not prove that it is a cause of NGU.

In the present study of *U urealyticum* in the urethra of healthy monogamous men, our aim was to try to establish: (a) whether *U urealyticum* is a stable, permanent inhabitant of healthy sexually active men or part of a transient urethral flora; and (b) whether or not there is any pronounced variation in the quantity of *U urealyticum* recovered from an individual over a period of time and if this variation is associated with any urethral signs and symptoms.

Patients and methods

STUDY POPULATION

Nineteen white men (aged 21-62 years) working at the Institute of Tropical Medicine in Antwerp were included in the study. They all claimed to be sexually active and were married; they denied a previous history of urethritis and were free of any infectious urinary tract disease as assessed by urine cultures for Gram-negative and Gram-positive bacteria.

CULTURAL TECHNIQUE

The first 10 ml of each man's overnight urine was collected weekly on the same day (Monday) for 17 consecutive weeks. A 0·2-ml quantity of fresh urine was cultured quantitatively in serial twofold or tenfold dilutions in bromothymolblue broth[7] (the former during the first 12 weeks of the study, the latter during the last five weeks). Colonial counts were performed on A7 agar[8] in tenfold dilutions of

Address for reprints: Dr P Piot, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium

Received for publication 5 September 1979

fresh urine. One drop (approximately 0·02 ml) of the pellet of centrifuged urine (at 400×g for 10 minutes) was also inoculated on to A7 medium and another drop was examined for the presence of leucocytes at ×400 magnification. All isolates were stored at −70°C in fetal calf serum without cloning.

SEROTYPING

Antisera to the eight serotypes of *U urealyticum*[9] were raised in rabbits as described by Shepard.[10] The growth inhibition (GI) test was performed as reported earlier,[11] except that A7 agar was used as the growth medium. The indirect immunoflourescence (IF) test using unfixed colonies on agar has already been described.[11] Antisera for the IF test were used at the following dilutions: 1/160 (types 1, 4, 5, and 8); 1/320 (type 7); and 1/640 (types 2, 3, and 6). Cultures were considered to be mixed when only part of the colonies on an agar block fluoresced with a particular antiserum; on other blocks from the same isolate some colonies strongly fluoresced with a different antiserum (an agar block in the test usually contained five colonies).

STATISTICAL METHODS

The statistical analyses were performed on the $\log_{10}$ of the first negative dilution for the titre and on the $\log_{10}$ of the number of leucocytes (plus 1 to avoid zero values to be transformed in log, this tranformation being the classical one to normalise the frequency data).

A one-way analysis of variance was used on the mean titres. The association between negative and positive and the number of leucocytes (<5, $\geqslant 5$) was tested by the χ^2 test for a 2×2 contingency table with Yates's correction.

Results

CULTURES

The 19 men were divided into four groups (A, B, C, and D) according to the rate of positive isolation. Twelve (63%) of them harboured *U urealyticum* in the urethra on at least one occasion, and in five of the culture-positive men ureaplasmas were isolated from more than 70% of the weekly specimens (table I). During the last five weeks of the study, five men in group A and one in group B had $>10^3$ colour-changing units (ccu) of *U urealyticum* in their urine on at least one occasion.

GEOMETRIC MEAN TITRES

The variation in the individual geometric mean titres with their 0·95 confidence interval for the first 12 weeks of the study is shown in fig 1. Values could fluctuate in the same patient and low values could immediately follow high values. Thus, those patients whose specimens frequently gave positive results had higher titres than those whose specimens less frequently gave positive results. A one-way analysis of variance showed a significant difference between the individual mean titre (F=7·045 with 11 and 12 degrees of freedom; $p<0·01$). A decomposition of the factorial sum of squares showed that this result was mostly due to the difference between group A

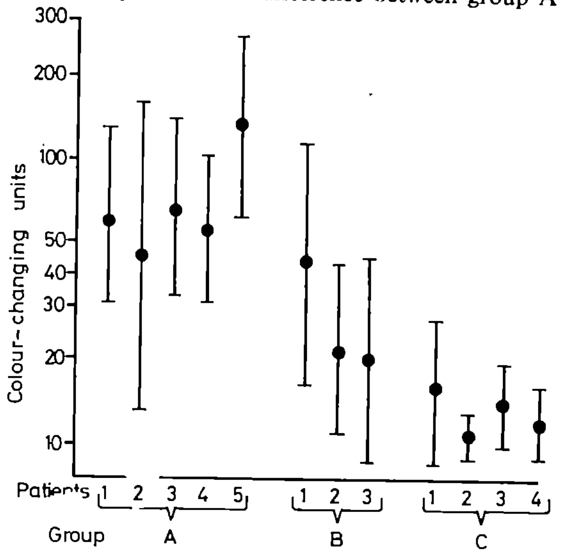


FIG 1 *Variation in individual geometric mean titres (colour-changing units) (with their 0·95 confidence intervals) of Ureaplasma urealyticum of patients grouped (A, B, C, and D) according to their positive isolation rate.*

TABLE I *Isolation rate, number of leucocytes, and titre of Ureaplasma urealyticum from specimens of first-voided urine from 19 men followed weekly for 17 weeks*

Patient group	*% Positive isolation (over 17 weeks)*	*No of patients*	*Geometric mean titre (ccu)*	*Geometric mean No of leucocytes*
A	>70	5	65·6	3·6
B	35–69	3	26·7	1·6
C	<35	4	12·8	2·0
D	0	7		1·3

ccu = Colour-changing units

and groups B and C (73% of the factorial sum of squares).

NUMBER OF LEUCOCYTES

The individual geometric mean numbers of leucocytes, with their 0·95 confidence interval, in culture-positive and culture-negative specimens (fig 2) showed great individual variation; there was no significant association between the number of leucocytes in the urinary sediment and the titre of *U urealyticum* present in the corresponding urine samples ($\chi^2_1 = 0·782$, P = 0·40), although culture-positive urine specimens tended to contain slightly more leucocytes than culture-negative specimens (mean of 3-4 leucocytes and 1-2 leucocytes respectively per microscopic field × 400). All samples contained less than 13 leucocytes per high-power field (HPF).

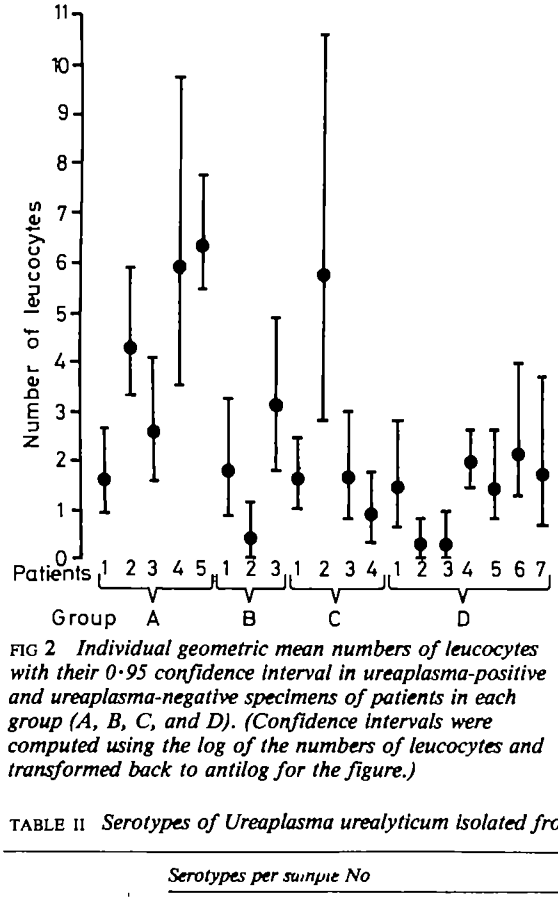


FIG 2 *Individual geometric mean numbers of leucocytes with their 0·95 confidence interval in ureaplasma-positive and ureaplasma-negative specimens of patients in each group (A, B, C, and D). (Confidence intervals were computed using the log of the numbers of leucocytes and transformed back to antilog for the figure.)*

SEROTYPES

Sixty-five strains isolated from nine men were serotyped by IF and 37 of these also by the GI test. The overall agreement between both tests was 81%, the discrepancy being caused exclusively by the inability of the GI test to type certain isolates. Table II shows the results obtained with the IF test. The predominant serotypes were 2 and 3, occurring in four and five men respectively. One person harboured type 7 only. Types 4, 5, and 6 were found occasionally in combination with types 2 and 3. Mixed isolates were found on at least one occasion in seven of nine men. The serotype present in each man was fairly constant, and in all but one case the same type was found in the first and the last culture tested.

A presumably primary infection with *U urealyticum* was diagnosed in patient 3 (table II), who had been culture-negative for the four preceeding weeks. Five days after extramarital intercourse *U urealyticum* serotype 3 was recovered from his first-voided urine specimen at a titre of 1/160 ccu and associated with 11 leucocytes per HPF × 400. For 10 days he complained of mild dysuria without discharge or any other signs of urethritis such as urinary mucous threads. This man remained infected with ureaplasmas for the remainder of the study, with titres varying between 1/20 and $1/10^5$ ccu and leucocytes between 4 and 11 per HPF × 400. No other patients had any symptoms or signs of urethritis.

Discussion

U urealyticum (notably types 2 and 3) appeared to be a part of the permanent urethral flora in at least one-

TABLE II *Serotypes of Ureaplasma urealyticum isolated from 12 samples of first-voided urine from eight healthy men*

Patient No	*Serotypes per sample No*											
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
1	3	3	3	3	3	3,6	3,6	*	*	*	*	*
2	2,7	2,3	3	3	2	2,3	2,3	3,5	2,5	2,5	2,5	NT
3	*	*	*	*	3	3	3	2	3,6	3,6	3,6	3
4	3,6	*	3,6	3,6	NT	NT	NT	NT	NT	NT	NT	NT
5	2	2	2,4	6	6	3	2	2,6	3	2	2	6
6	2	2	2,3	2	2	2	2	2	2	2,6	2	2
7	NT	2,6	2,6	*	3,6	3	NT	2	2	NT	2	6
8	*	*	3	*	*	3	NT	NT	*	*	*	*

*No *U urealyticum* recovered
NT = not tested

third of the participants in this study, as suggested by the continuous isolation of the same serotype. This supports the observations on men working in the Antarctic without exposure to heterosexual contact for up to 31 months.[12,13] Most of the men in these studies had a persistent infection with *U urealyticum* (non-typed), which could be eradicated for one year or more by a five-day course of tetracycline.

As in the study by Holmes,[12] ureaplasmas were inconsistently recovered from some men, but in our survey it is not clear whether this was the result of occasional reinfection by their sexual partner or of a lack of sensitivity of the isolation methods used, which may not detect consistently fastidious strains of *U urealyticum*.

The significantly higher geometric mean titre of *U urealyticum* in patients with more than 70% of positive culture results compared with the other culture-positive men in this study possibly reflects the existence of a subgroup permanently heavily colonised with ureaplasmas and of a group harbouring fewer organisms, which cannot always be found by the isolation methods used. Similarly, the occasional detection of mixed infections in colonised patients may be explained by the occurrence of fastidious strains or of low numbers of *U urealyticum*, although failure to detect mixed cultures may be due to the limited number of colonies which are stained in the IF test.

This study confirms Bowie's[3] findings on the number of polymorphonuclear leucocytes in the first-voided urine of men without urethritis. By similar methods, we never found >12 leucocytes per HPF in our specimens from healthy men whereas Bowie[3] considered >15 leucocytes per HPF as normal. In the absence of cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* this finding should be of value in the diagnosis of NGU, particularly in patients without urethral discharge.

Although a significant correlation exists between a first episode of chlamydia-negative NGU and a ureaplasmal titre of $>10^3$ ccu in a first-voided urine sample,[1] it is clear from our results that this is not a directly causal association. Except for patient 3, none of the men studied developed NGU or related signs and symptoms in association with pronounced increases in ccu of *U urealyticum* in their urine. Moreover, prevalence studies in more heterogeneous venereal disease clinic populations have failed to confirm Bowie's observation[14] (Piot, unpublished data) in men experiencing a first episode of NGU. One man with presumably his first infection developed dysuria without a concomitant increase in urinary leucocytes. It is not clear whether the association of ureaplasmas with dysuria in this case is coincidental or causal. Our study group did not include enough men experiencing a first infection with *U urealyticum*. Considering the high prevalence of *U urealyticum* colonisation in sexually active people it is highly improbable that a simple primary infection with any type would cause NGU. Cohort studies to correlate the acquisition of ureaplasmas with NGU are extremely difficult to organise and would be severely handicapped by the lack of sensitive and specific serological tests.

It still remains possible that only certain serotypes or biotypes are pathogenic for the urethra, a hypothesis which has not been tested in a comprehensive study including cultures for chlamydia. This approach seems the most reasonable one at the moment, together with the search for a good experimental animal model as attempted by Bowie and coworkers.[15]

References

1. Bowie WR, Wang SP, Alexander ER, *et al.* Etiology of nongonococcal urethritis. Evidence for *Chlamydia trachomatis* and *Ureaplasma urealyticum*. *J Clin Invest* 1977;**59**:735-42.
2. Shepard MC. Quantitative relationship of *Ureaplasma urealyticum* to the clinical course of nongonococcal urethritis in the human male. *In:* Bové JM, Duplan JF, eds. *Les Mycoplasmes de l'Homme, des Animaux, des Végétaux, et des Insectes.* Paris: Inserm, 1974:375-9.
3. Bowie WR. Comparison of Gram stain and first-voided urine sediment in the diagnosis of urethritis. *Sex Transm Dis* 1978;**5**:39-42.
4. Coufalik ED, Taylor-Robinson D, Csonka GW. Treatment of nongonococcal urethritis with rifampicin as a means of defining the role of *Ureaplasma urealyticum*. *Br J Vener Dis* 1979;**55**:36-43.
5. Rodermund OE, Jänsch HH. Zur frage der bedeutung von mykoplasmen in der atiopathogenese nicht gonorrhoischer urethritiden des mannes. *Hautarzt* 1974;**25**:597-602.
6. Taylor-Robinson D, Csonka GW, Prentice MJ. Chlamydial and ureaplasma-associated urethritis. *Lancet* 1977;i:903.
7. Robertson JA. Bromothymolblue broth: improved medium for detection of *Ureaplasma urealyticum* (T-strain mycoplasma). *J Clin Microbiol* 1978;**7**:127-32.
8. Shepard MC, Lunceford CD. A differential agar medium (A7) for identification of *Ureaplasma urealyticum* (human T mycoplasma) in primary cultures of clinical material. *J Clin Microbiol* 1976;**3**:613-25.
9. Shepard MC, Lunceford CD, Ford DK, *et al.* *Ureaplasma urealyticum* gen nov, sp nov: proposed nomenclature for the human T (T-strain) mycoplasma. *Internat J Syst Bacteriol* 1974;**24**:160-71.
10. Shepard MC, Lunceford CD. Serological typing of *Ureaplasma urealyticum* isolates from urethritis patients by an agar growth inhibition method. *J Clin Microbiol* 1978;**8**:566-74.
11. Piot P. Comparison of growth inhibition and immunofluorescence test in serotyping clinical isolates of *Ureaplasma urealyticum*. *Br J Vener Dis* 1977;**53**:186-9.
12. MacLeod AD, Furr PM, Taylor-Robinson D. Prolonged eradication of urogenital mycoplasmas after administration of tetracycline in men in the Antarctic. *Br J Vener Dis* 1976;**52**:337-40.
13. Holmes MJ, Furr PM, Taylor-Robinson D. The persistence of mycoplasmas in the urogenital tract of men in the Antarctic. *J Hyg (Cambridge)* 1974;**72**:355-63.
14. Taylor-Robinson D, Evans RT, Coufalik ED, *et al.* *Ureaplasma urealyticum* and *Mycoplasma hominis* in chlamydial and non-chlamydial nongonococcal urethritis. *Br J Vener Dis* 1979;**55**:30-35.
15. Bowie WR, Digiacomo, RF, Holmes KK, Gale JL. Genital inoculation of male *Macaca fascicularis* with *Neisseria gonorrhoeae* and *Ureaplasma urealyticum*. *Br J Vener Dis* 1978;**54**:235-8.

Br J Vener Dis 1980;56:173-7.

Venereal diseases in the islands of the North Pacific

R R WILLCOX
From St Mary's Hospital, London

SUMMARY Apart from the Japanese islands, and those of Karabati (lately Gilbert Islands), which lie just north of the equator, the islands of the northern Pacific Ocean are either American owned or otherwise administered. Even the Japanese islands were controlled by the USA for varying numbers of years after the second world war. Venereal disease statistics from Guam, the Trust Territory of the Pacific Islands, and the Gilbert Islands have been collated by the South Pacific Commission and will be presented in a second paper. Those from the Hawaiian Islands (the fiftieth state of the USA) are published by the United States Public Health Service and include those from Honolulu, the capital.

While the rates per 100 000 for both syphilis and gonorrhoea are lower than those for the USA as a whole, the trends since 1970 have been less satisfactory in the state of Hawaii than for the whole of the United States. While the disturbing increasing incidence of primary and secondary syphilis was checked in 1977, that of gonorrhoea has continued to rise. The number of cases of gonorrhoea also increased in Guam and the Trust Territory of the Pacific Islands but there has been a recent fall from earlier peak figures. The pattern of venereal disease in the most developed Pacific islands is thus gradually approaching what may be expected elsewhere in modern western society and it would seem logical to expect that this trend will continue.

Introduction

HISTORICAL ASPECTS

The Pacific Islands have had a mixed and varied past, being discovered, conquered, or otherwise acquired and later administered directly or indirectly under the mandate or trusteeship of the League of Nations after the first world war or of the United Nations after the second world war, by one or more of a number of countries including Australia, Britain, Chile, Ecuador, France, Germany, Holland, Japan, New Zealand, Portugal, Spain, and the USA. In recent years increasing numbers are achieving independence, particularly those south of the equator.

The changing past can well be illustrated by the administration of the Mariana Islands. After occupation by Spain they were sold to Germany in 1899, placed under Japanese mandate by the Treaty of Versailles in 1919, and after the second world war became part of the Pacific Islands Trust Territory of the USA with the ultimate goal of US commonwealth status.

POLITICAL ASPECTS

The present political attachments of the Pacific islands north of the equator are shown in table I.

Thus the relatively few Pacific islands north of the equator are orientated towards the USA. Even the Japanese islands (table I) were controlled by the USA after the second world war, since when their administration has gradually been restored to Japan.

DEMOGRAPHIC ASPECTS

Hawaiian group

The Hawaiian islands are a mixture of high islands, mountainous and volcanic in origin (in some cases volcanoes are still active) with an external coral-reef-bounded lagoon, or low coral atolls with an internal lagoon. The centre of the North Pacific Ocean is occupied by the long chain of mixed islands of the Hawaiian group (population 838 000, with the modern sophisticated capital, Honolulu, on the island of Oahu), which became the fiftieth state of the USA in 1959. Midway Island lies near its western extremity.

---

*Paper presented at a meeting of the Medical Society for the Study of Venereal Diseases, Lubeck, West Germany, May 1979

Address for reprints: Dr R R Willcox, Tideway, Lonsdale Road, London SW 13

Received for publication 21 August 1979

TABLE I *Political attachments of Pacific Islands north of the equator*

Political attachment	*Pacific Islands*
Independent	Part of Karabati, lately the Gilbert Islands (British), including Tarawa and certain Line Islands (Washington, Fanning, and Christmas Islands)
Japan	Rukyu Islands (including Okinawa) Bonin and Volcano Islands (including Iwo Jima) Ganges Island Marcus Island
USA	Hawaiian Islands Midway Island Certain Line Islands (Palmyra, Kingman, Jarvis) Johnston Wake Guam US Trust Territory of Pacific Islands (Marshalls, Carolines and remaining Marianas)

Japanese islands

On the Asian side a chain of volcanic islands, the Ryukyu archipelago, extends south-eastwards towards Taiwan. These comprise 848 square miles of land and are relatively heavily populated (977 000 inhabitants in 1977).

Guam

Due south of Japan are the 15 Bonin Islands, with 40 square miles and a population of only 203 in 1965, and the three Volcano Islands, which are also Japanese. These groups are geographically part of a north-south archipelago, which extends further to include the Marianas; Guam, the 209 square miles of which were seceded to the USA by Spain in 1898 and which had a population in 1979 of 90 000, is the southernmost member.

Trust Territory of Pacific Islands

The remaining Marianas and the more southerly Caroline and Marshall groups of islands form the US Trust Territory of the Pacific Islands, with a population of 133 000 on 707 square miles of land. The 1350 coral islets of the Caroline Islands and the 34 coral atolls of the Marshall Islands stretch from west to east for 4000 miles between 0° and 10° north of the equator, leaving only Johnston Island to bridge the long gap of ocean north-east to Hawaii.

Gilbert Islands

Further south straddling the equator for a similar distance are some members of the Gilbert Islands (266 square miles, population 56 000). These include the capital, Tarawa (recently the scene of a cholera epidemic), and some of the Line Islands (for example, Christmas Island, the site of nuclear tests between 1957 and 1964). Others lie south of the equator, including Ocean Island (Banaba) and all of the Phoenix Islands.

Described in the popular press as "33 coral atolls scattered across two million square miles of Pacific" the Gilbert Islands achieved independence on 11 July 1979 and were renamed Karabati.

COMMUNICATIONS

Sixteen airlines serving the Pacific Islands north of the equator are listed in table II; all but three routes pass through Honolulu. Other internal American airlines connect Honolulu with the mainland of the USA.

East-west communications lie through Guam and Honolulu with a linking island-hopping operation by way of Johnston Island through the Trust Territory of the Pacific Islands (with stops at Majuro and Kwajalein in the Marshall Islands, Ponape and Truk in the Carolines). North-south communications are mainly through Honolulu or Nauru, which lies only a few (26 miles) south of the equator (figure).

TABLE II *Airlines* serving the Pacific Islands north of the equator*

External	*Internal*
Air New Zealand (CTE)	Air Nauru (ON)†
Canadian Pacific Air (CP)	Air Pacific (FJ)†
China Airlines (CI)	Continental Air Micronesia (CO)
Japan Airlines (JL)	Hawaiian Airlines (HA)
Korean Airlines (KE)	Island Air (IL)*†
North-West Orient Airlines (NW)	
Philippine Airlines (PR)	
Pan American World Airways (PA)	
Qantas Airways (QF)	
Singapore Airlines (SQ)	
Western Airlines (WA)	

*All but those marked † use Honolulu; letters in parentheses are codes[11]

CONSEQUENCES OF WESTERNISATION

The early voyagers brought many infectious diseases for example, (influenza, measles, pertussis, and some sexually transmitted conditions)—often with drastic consequences—to add to the usual parasitic and other communicable conditions endemic in the then undeveloped tropical areas.

A further marked change has taken place following the more recent vastly increased expansion of air travel and its accompanying tourism and other economic developments. For decades this development has been most advanced in the Hawaiian Islands; Honolulu, with its many modern buildings, may be equated with Miami. However, large hotels are now also appearing elsewhere (there is a Guam

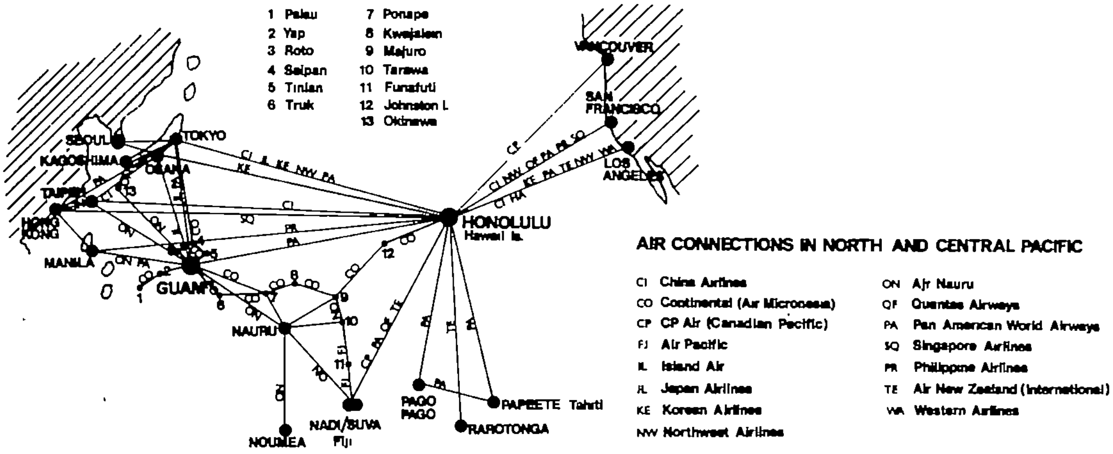


FIG *Air connections in North and Central Pacific.*

Hilton and a Continental Hotel in Guam and the Palau (Caroline) Islands).

These changes have produced a life style, which, for many of the local inhabitants of the Pacific Islands, has proved far from beneficial. Although a number of communicable diseases are now well controlled (for example, yaws) there has been a recent upsurge of venereal infections and degenerative diseases, such as diabetes, hypertension, and obesity.

For example, in the once phosphate-rich eight square miles of Nauru, which was successively governed by Germany, Britain, and Australia before becoming independent in 1968, there is an annual income of US$30 000 per head, but 31% of its 7300 population now have diabetes.[1 2]

Venereal diseases statistics

AVAILABLE DATA

No data are readily available for the Japanese islands. Those for Hawaii and Honolulu are published regularly by the United States Public Health Service together with those for other American states. Figures for the other territories are collated and published by the World Health Organisation and by the South Pacific Commission and are subject to the usual variables—for example, the reliability of diagnosis, intensity of medical services, degree of support by pathology services, extent of reporting, and indeed the vagaries inherent in statistical assembly itself.

DATA FROM HAWAII

Gonorrhoea

In the state of Hawaii the rates per 100 000 for gonorrhoea have consistently increased from 177·4 in 1970[3] to 398·2 in 1977[4] (table III). Both these figures were lower than those for the United States as a whole, which were 287·1 per 100 000 in 1970 and 465·9 per 100 000 in 1977 (the figures for 1977 were for the calendar not the fiscal year as previously). Nevertheless the rate for Hawaii had more than doubled during this time compared with only a 1·6-fold increase for the whole of the USA.

The male-to-female ratio for gonorrhoea has increased from 1·04 : 1 in 1975 to 1·38 : 1 in 1977 (table IV). The national average for the United States in 1977 was 1·48 : 1.[4]

TABLE III *Venereal disease in Hawaii*[3-5]

	Fiscal years									
	1970		*1972*		*1974*		*1976*		*1977*	
Venereal diseases	*No**	*Rate**	*No*	*Rate*	*No*	*Rate*	*No*	*Rate*	*No*	*Rate*
Syphilis (total)	66	9·0	78	10·6	69	8·9	133	16·5	72	8·6
Syphilis										
Primary and secondary	8	1·1	22	3·0	38	4·9	73	9·1	36	4·3
Gonorrhoea	1265	177·4	1612	218·4	2182	281·2	2508	311·2	3337	398·2
Other	1	0·1	0	0	1	0·1	0	0	0	0

*Cases and Rates per 100 000 population

TABLE IV *Male-to-female ratio for venereal diseases in Hawaii*

Venereal disease	*1975**			*1976**			*1977†*		
	M	*F*	*Ratio*	*M*	*F*	*Ratio*	*M*	*F*	*Ratio*
Syphilis									
Primary and secondary	37	3	12·3	69	4	17·3	29	7	4·1
Gonorrhoea	1194	1148	1·04	1333	1176	1·13	1936	1401	1·38

*Fiscal year[5]
†Calendar year[4]

The proportion of gonorrhoea treated in the capital, Honolulu, in 1977, the approximate population of 368 000 which represented 44% of that of Hawaii as a whole, was much higher at 70%, a phenomenon of concentration of venereal disease in the more urban areas which has been apparent for a long time in other large cities of the USA and elsewhere.

Syphilis

Cases and rates: The total rate for syphilis increased from 9·0 per 100 000 in the fiscal year, 1970, to 16·5 per 100 000 in 1976 but fell to 8·6 per 100 000 in the calendar year, 1977 (table III). For the USA as a whole the comparable figures were much higher at 44·0 per 100 000 in 1970, 36·3 in 1976, and 30·0 in 1970.[5]

The notably increasing rates for total syphilis in Hawaii were due to an increasing number of cases of primary and secondary syphilis, which increased from eight (12·1%) cases in 1970 to 73 (54·9%) cases in 1976 with a slight fall to 36 (50%) cases in 1977. In the United States as a whole, the percentage of early cases compared with all cases of syphilis rose from 23·0% in 1970 to 32·5% in 1976 and 33·1% in 1977.[4 5]

For primary and secondary syphilis (table III) the rates for Hawaii increased from 1·1 per 100 000 in 1970, when the rate was 10·1 per 100 000 in the USA, to 9·1 per 100 000 in 1976, when the rate had been reduced to 9·5 per 100 000 in the USA as a whole.[5] Thus, for both syphilis and gonorrhoea the trends have been less satisfactory in Hawaii than the average for the United States as a whole.

Male-to-female ratio: On the basis of relatively few cases the male-to-female ratio in Hawaii has been appreciably higher for primary and secondary syphilis than for gonorrhoea (table IV). During the years when the rates for primary and secondary syphilis were rising sharply the ratio was high, reaching 17·3 : 1 in 1976, but fell with the reduction of cases in 1977. Various factors—such as more intensive contact-tracing for syphilis and more homosexual infections—could explain the difference.

Proportion treated in Honolulu: The proportion of cases of primary and secondary syphilis treated in Honolulu decreased between 1973 and 1977. This trend was not so striking, however, for cases of gonorrhoea (table V). The difference could again be attributable to differing degrees of emphasis on contact-tracing for the two diseases.

TABLE V *Proportion of cases of syphilis and gonorrhoea treated in Honolulu compared with Hawaii as a whole*

No of cases	*1973**		*1976**		*1977†*	
	No	*%*	*No*	*%*	*No*	*%*
Syphilis (primary and secondary)						
Hawaii (total)	48		73		36	
Honolulu	42	87·5	56	76·7	19	52·8
Gonorrhoea						
Hawaii (total)	1756		2508		3337	
Honolulu	1239	70·6	1692	67·5	2335	70·0

*Fiscal year
†Calendar year

OTHER SEXUALLY TRANSMITTED DISEASES

Chancroid, lymphogranuloma venereum, and granuloma inguinale, which are classified as "other VD" in USA statistics, are virtually non-existent in Hawaii (table III). Nongonococcal urethritis, which is not reportable on the other hand, is extremely common. Kuberski *et al*[6] noted that 75% of cases of urethritis seen in the Hawaii State Venereal Diseases Clinic in Honolulu were nongonococcal in origin; indeed nearly 70% of all male patients attending the clinic had this condition. Seventy-three per cent of a selected group of patients had antibodies to herpes virus hominis, 66% to chlamydia, and 72% to cytomegalovirus. Moreover, *Trichomonas vaginalis* was isolated from 24% of female patients.[7]

GUAM AND THE PACIFIC ISLANDS

The World Health Organisation[8] (Causse, G, unpublished data), in its figures for Oceania, provides data on Guam and the Trust Territory of the Pacific Islands. No data are forthcoming for syphilis for either of these territories, but the number of cases of gonococcal infection are shown in table VI.[10] The number of cases increased in both

TABLE VI *Number of cases of gonococcal infection in Guam and the Trust Territory of the Pacific Islands*[9]

Year	*Guam*	*TTPI*
1955		599
1966		703
1967	236	488
1968	279	497
1969		400
1970	231	423
1971	191	661
1972	459	858
1973	422	858
1974	344	629
1975	426	532
1976	354	413

TTPI = Trust Territory of the Pacific Islands

territories in the early 1970s but more recently the number fell from the peak figures obtained.

Figures for these territories, and also for the Gilbert Islands, are collated in the annual reports of the South Pacific Commission as they form part of its northern region. They will therefore also be considered in this context in a companion paper.

Nevertheless, some earlier data supplied to the South Pacific Commission of the Trust Territory of the Pacific Islands confirm the absence of syphilis before 1972 (although very few cases have been reported since[13]) and also provide figures for gonorrhoea before 1971 with a breakdown according to Districts. Palau in the Caroline Islands was the district with the highest number of cases (table VII). In 1972 efforts to control gonorrhoea in that country were being intensified by such means as the use of screening by culture of high-risk groups. Because of the insular situation and relatively small number of cases it seemed possible not only to control, but to eradicate, gonorrhoea in the Trust Territory using available technology. Predictably the application of this programme resulted initially in an increase in the number of cases being diagnosed before the number began to decline.

TABLE VII *Number of cases of gonorrhoea in Trust Territory of Pacific Islands*[12]

		No of cases		
Territory	*Population (1973)*	*1961*	*1966*	*1971*
Mariana Islands	14 333*	0*	25	24
Caroline Islands				
Palau	12 673	96	266	338
Yap	7870	8	97	124
Ponape	23 252	86	146	72
Truk	31 609	50	50	8
Marshall Islands	25 045	31	119	95
Total	115 251†	271	703	661

*Rota only
†Includes 469 persons not allocated to districts

Thanks are expressed to Dr G Causse of the World Health Organisation, Geneva; Dr T Kuberski of the South Pacific Commission, Noumea, New Caledonia; and Dr P J Wiesner, Centre for Disease Control, Atlanta, Georgia, USA for kindly making available the statistics on which this paper was based.

References

1. Zimmet P. Diabetes in Pacific populations—price for westernisation. *Proceedings of the Sixth Asia and Oceania Congress of Endocrinology, Singapore,* 1978;256-65.
2. Zimmet P. Regional workshop on cholera and other epidemic diseases in the Pacific, Noumea, New Caledonia, 24-28 April 1978. *Report of the South Pacific Commission* 1978;62-3.
3. Center for Disease Control. *VD Fact Sheet 1970.* Washington DC: United States Public Health Service, 1971.
4. Center for Disease Control. *STD Fact Sheet,* 34th edition, Washington DC: United States Public Health Service, 1979.
5. Center for Disease Control. *VD Fact Sheet 1976,* 33rd edition. United States Public Health Service, 1977.
6. Kuberski T, Perry S, Ohye R, Wiebenga N. Non-gonococcal urethritis in Hawaii. *Hawaii Med J* 1977;36:103-8.
7. Kuberski T. Evaluation of the indirect haemagglutination technique for the study of *Trichomonas vaginalis* infections, particularly in men. *Sex Transm Dis* 1978;5:97-102.
8. World Health Organisation. *World Health Stat Rep* 1975;**28:**118, 125.
10. Willcox RR. Regional workshop on cholera and other epidemic diseases in the Pacific, Noumea, New Caledonia, 24-28 April. *Report of the South Pacific Commission* 1978:63-6.
11. *Official Airline Guide.* Hinsdale, Illinois: Reuben H Donnelley Corp, 1978.
12. South Pacific Commission. *Annual Report of the South Pacific Epidemiological and Health Information Service for the Year 1978.* Noumea, New Caledonia: South Pacific Commission, 1979.
13. Willcox RR. Venereal diseases in the islands of the South Pacific. *Br J Vener Dis* (In press).

Br J Vener Dis 1980;**56**:178-81.

Sexually transmitted diseases

Extract from the *Annual Report of the Chief Medical Officer of the Department of Health and Social Security for the Year 1978*[1]

The principal characteristics of the year under review have been the continued increase in new cases of non-specific genital infection, genital candidosis, and genital herpes simplex. There has also been an increase in the number of cases of both early and late syphilis, but the incidence of gonorrhoea appears to have levelled out and there was actually a slight reduction in the number of reported cases in both sexes.

The number of new patients attending the clinics increased by 2% to 518 839. There was also a 5% increase to 96 769 in the number of patients attending who were found to be healthy and non-infected, indicating that the public continues to use the services of the clinics for counselling on a wide variety of sexual problems and conditions which are not transmitted by sexual contact. The incidence of sexually transmitted diseases which are symptomless is increasing and there is some indication that people who have been at risk of infection are more inclined to attend for a check-up to allay their anxiety.

Syphilis, gonorrhoea, and chancroid

SYPHILIS

Table I shows an increase in the incidence of early syphilis of 6·4% (5·4% in men and 11·8% in women) but the figures for primary and secondary syphilis show little change. Late syphilis shows a 15·8% increase overall. The rate per 100 000 population is given in table II.

GONORRHOEA

Total cases of gonorrhoea showed a decrease of 2·8% (men 2·5%; women 3·3%) (tables I and II). Penicillin-resistant β-lactamase-producing strains of gonococci continue to be imported into this country from West Africa and South-east Asia in small numbers. Their diffusion throughout the United Kingdom has been prevented by prompt recognition, correct treatment, energetic contact-tracing, and surveillance to ensure that a complete cure has occurred. Continuous vigilance is essential if gonorrhoea is to continue to be controlled in the United Kingdom.

CHANCROID

There was a slight increase in the number of cases in both sexes (tables I and II).

Other sexually transmitted diseases

NON-SPECIFIC GENITAL INFECTION

Cases of non-specific genital infection have increased

TABLE I *Cases of syphilis, gonorrhoea and chancroid reported in England for the year ending 30 June 1978, with the revised figures for the year ending 30 June 1977 in parentheses (for the incidence rates per 100 000 population see table II†)*

	Total No of cases		*Men*		*Women*	
Syphilis						
Early	2755	(2590)	2300	(2183)	455	(407)
Primary and secondary only	1747	(1733)	1499	(1486)	248	(247)
Late	1427	(1232)	976	(828)	451	(404)
Congenital	155	(144)	51	(57)	104	(87)
Gonorrhoea						
All forms	57 501	(59 155)	36 266	(37 204)	21 235	(21 951)
Post-pubertal gonorrhoea						
All ages	57 465	(59 116)	36 254	(37 192)	21 211	(21 924)
Under 16 years	577	(568)	149	(112)	428	(456)
16-19 years	11 304	(11 836)	4558	(4782)	6746	(7054)
20-24 years	19 317	(19 903)	11 767	(12 196)	7550	(7707)
25-34 years*	18 720	(19 109)	13 589	(13 823)	5131	(5286)
35-44 years*	5753	(5872)	4687	(4729)	1066	(1143)
45 years and over*	1794	(1828)	1504	(1550)	290	(278)
Chancroid	49	(42)	43	(39)	6	(3)

*Only one group (25 years and over) was collected before 1977.

†Note: in the original report this should read, "Table 5·3" and not "Table 4·3."

TABLE II *The venereal diseases: new cases per 100 000 population, by age, seen at hospital clinics in England 1974–78*

	Years ending 30 June														
	1974			*1975*			*1976*			*1977*			*1978*		
Disease	*Men*	*Women*	*Total*	*Men*	*Women*	*Total*	*Men*	*Women*	*Total*	*Men*	*Women*	*Total*	*Men*	*Women*	*Total*
Early syphilis															
All ages	8·57	1·46	4·92	8·44	1·48	4·87	8·86	1·50	5·08	9·67	1·71	5·59	10·18	1·91	5·94
Early syphilis (primary and secondary only)															
All ages	6·30	1·01	3·59	6·12	0·93	3·46	6·40	0·87	3·56	6·58	1·04	3·74	6·64	1·04	3·77
Under 16 years*	0·05	0·05	0·05	0·05	0·02	0·04	0·07	0·09	0·08	0·04*	0·04*	0·04	0·19	0·09*	0·14
16-19 years	7·01	3·55	5·32	6·54	3·76	5·18	7·52	3·30	5·46	5·72	4·56	5·15	7·09	4·70	5·93
20-24 years	18·16	4·26	11·29	18·02	4·60	11·42	19·35	3·46	11·58	17·76	5·02	11·56	19·52	4·83	12·36
25 years and over	7·52	0·81	3·98	7·26	0·65	3·77	7·40	0·68	3·85	8·01	0·66	4·13	7·68	0·66	3·97
Late syphilis															
All ages	3·51	1·51	2·48	3·68	1·73	2·68	3·87	1·76	2·79	3·67	1·70	2·66	4·32	1·90	3·08
Congenital syphilis															
All ages	0·21	0·42	0·32	0·32	0·46	0·39	0·28	0·33	0·30	0·25	0·37	0·31	0·22	0·44	0·33
Gonorrhoea (post-pubertal)															
All ages	169·60	88·92	128·19	163·67	87·62	124·66	163·91	89·60	125·79	164·70	92·23	127·54	160·55	89·23	124·00
Under 16 years	2·30	8·33	5·23	1·89	8·26	4·99	1·87	8·57	5·13	1·98	8·52	5·16	2·64	8·00	5·25
16-19 years	361·92	535·55	446·72	340·26	513·59	424·85	331·52	513·05	420·19	329·41	510·53	417·73	313·91	488·13	399·01
20-24 years	769·43	469·03	620·96	753·58	472·99	615·66	728·41	475·72	604·92	736·92	490·17	616·71	711·00	480·18	598·60
25 years and over	152·00	42·12	93·95	146·11	41·03	90·42	147·97	42·73	92·40	145·38	43·36	91·52	143·05	41·94	89·67
Chancroid															
All ages	0·16	0·02*	0·09	0·18	0·03*	0·10	0·27	0·03*	0·15	0·17	0·01*	0·09	0·19	0·02*	0·11

*These rates were based on fewer than 10 events and consequently their reliability as a measure may be affected.

TABLE III *Other sexually transmitted diseases reported in the year ending 30 June 1978 with the revised figures for the year ending 30 June 1977 in parentheses (for the incidence per 100 000 population see table IV*)*

	Total No of cases		*Men*		*Women*	
Lymphogranuloma venereum	27	(37)	26	(28)	1	(9)
Granuloma inguinale	14	(14)	11	(10)	3	(4)
Non-specific genital infection (NSGI)	96 564	(94 461)	76 826	(74 295)	19 738	(20 166)
NSGI with arthritis	498	(591)	463	(549)	35	(42)
Trichomoniasis	19 943	(19 992)	1709	(1705)	18 234	(18 287)
Candidosis	38 465	(36 805)	7808	(6794)	30 657	(30 011)
Scabies	2237	(2231)	1767	(1765)	470	(466)
Pediculosis pubis	6206	(5850)	4347	(4093)	1859	(1757)
Genital herpes	7992	(7334)	5080	(4696)	2912	(2638)
Genital warts	23 332	(22 371)	15 113	(14 637)	8219	(7734)
Genital molluscum	933	(901)	636	(603)	297	(298)
Other treponemal diseases	1140	(1154)	740	(738)	400	(416)
Other conditions requiring treatment in a centre	44 194	(39 897)	28 399	(25 852)	15 795	(14 045)
Other conditions not requiring treatment in a centre	96 769	(91 899)	60 873	(57 023)	35 896	(34 876)
Other conditions referred elsewhere	1961	(1887)	1172	(1143)	789	(744)

*Note: in original report this should read, "Table 5·4" and not "Table 4·4."

TABLE IV *Other sexually transmitted diseases and other conditions: new cases per 100 000 population at all ages seen at hospital clinics in England 1974-78*

	Years ending 30 June														
	1974			*1975*			*1976*			*1977*			*1978*		
Disease	*Men*	*Women*	*Total*	*Men*	*Women*	*Total*	*Men*	*Women*	*Total*	*Men*	*Women*	*Total*	*Men*	*Women*	*Total*
Lymphogranuloma venereum	0·15	0·03*	0·03	0·18	0·03*	0·10	0·14	0·01*	0·07	0·12	0·04*	0·08	0·12	0·00*	0·06
Granuloma inguinale	0·04	0·02*	0·03	0·02*	0·03*	0·02	0·05	0·02*	0·03	0·04	0·02	0·03	0·05	0·01*	0·03
Non-specific genital infection	307·95	63·58	182·50	306·90	65·87	183·25	310·35	73·60	188·91	329·01	84·84	203·79	340·22	83·04	208·33
Non-specific genital infection with arthritis	1·87	0·08	0·95	1·92	0·14	1·01	2·07	0·01	1·06	2·43	0·18	1·28	2·05	0·15	1·07
Trichomoniasis	6·88	74·02	41·35	6·42	74·96	41·58	6·81	76·23	42·42	7·55	76·93	43·13	7·57	76·71	43·03
Candidosis	22·84	115·53	70·42	23·71	116·83	71·48	27·68	125·84	78·02	30·09	126·26	79·40	34·58	128·97	82·99
Scabies	9·64	2·13	5·79	10·20	2·01	6·00	9·41	2·19	5·17	7·82	1·96	4·81	7·82	1·98	4·83
Pubic lice (pediculosis pubis)	15·37	5·22	10·16	16·07	5·78	10·80	16·07	6·58	11·21	18·12	7·39	12·62	19·25	7·82	13·39
Herpes simplex	15·28	6·88	10·97	16·77	7·86	12·20	19·10	9·83	14·35	20·80	11·10	15·82	22·50	12·25	17·24
Warts (condylomata acuminata)	53·11	26·02	39·20	56·58	27·98	41·91	60·31	30·71	45·13	64·82	32·54	48·26	66·93	34·58	50·34
Molluscum contagiosum	2·02	0·87	1·43	2·26	0·79	1·50	2·53	1·08	1·78	2·67	1·25	1·94	2·82	1·25	2·01
Other treponemal diseases	2·64	1·43	2·02	3·17	1·59	2·36	2·94	1·60	2·25	3·27	1·75	2·49	3·28	1·68	2·46
Other conditions requiring treatment in a centre	112·19	44·55	77·47	111·39	49·70	79·74	112·32	55·75	83·30	114·48	59·09	86·08	125·76	66·45	93·35
Other conditions not requiring treatment in a centre	240·57	132·52	185·10	232·69	132·61	181·35	237·48	136·00	185·43	252·54	146·72	198·27	269·58	151·01	208·77
Other conditions referred elsewhere†							2·40†	1·33†	1·85†	5·06	3·13	4·07	5·19	3·32	4·23

*Rates based on fewer than 10 events and consequently their reliability as a measure may be affected.
†Figures collected from January 1976.

by 2·2% (3·4% increase in men; 2·1% decrease in women) (tables III and IV).

CANDIDOSIS

Cases of candidosis have increased in both sexes, more particularly in men (14·9% increase) (tables III and IV).

GENITAL HERPES

This has increased in both sexes (tables III and IV).

Contact-tracing

Considerable progress continues to be made in contact-tracing and more workers and improved facilities are now available in clinics throughout the country. The Society of Social Health Workers in Venereology is now well established and holds an annual conference in a major centre in the United Kingdom. The last meeting was held in Liverpool and was well attended. Efforts are continuing to provide training opportunities for this group of workers.

Staffing

The number of hospital medical staff engaged in genitourinary medicine in England and Wales at 30 September 1978 totalled 203 (185 whole-time equivalents (wte) compared with 211 (188·6 wte) on 30 September 1977). The 1978 figures included 101 (95·1 wte) consultants, 36 (33·7) senior registrars, and 35 (33·5) registrars compared with 106 (99 wte) consultants, 35 (32·6 wte) senior registrars, and 32 (30·2 wte) registrars at 30 September 1977. At 30 September 1978, in addition to the above, there were 17 (4·8 wte) hospital practitioners and 144 (33·3 wte) staff holding sessional appointments under paragraph 94 of the Terms and Conditions of Service.

The Specialist Advisory Committee in Genitourinary Medicine of the Royal College of Physicians recommends that applicants for consultant posts should possess a suitable higher qualification—usually the Membership of the Royal College of Physicians—and have completed four years of specialist training at senior registrar level. Because recruitment in the specialty still causes problems in some areas of the country, the Specialist Advisory Committee may sometimes give accreditation to trainees from other branches of medicine whose previous experience has been carefully assessed and found to be acceptable.

Recruitment of suitable male nurses has been inadequate in recent years and retirements have left some important posts unfilled. It is hoped that the highly successful post-registration courses organised by the Joint Board of Clinical Nursing Studies will help to solve this problem by attracting younger nurses to the specialty.

Education and training

The first professor of genitourinary medicine in the world, Professor Michael Adler, occupied the chair created at the Middlesex Hospital Medical School of London University on 1 January 1979 and is now establishing the academic department.

The 29th General Assembly of the International Union against the Venereal Diseases and Treponematoses took place in Leeds in May 1978. There were over 200 delegates from 25 different countries in all parts of the world. Dr R D Catterall, consultant adviser in genitourinary medicine to the DHSS, was elected President of the International Union for the next five years.

Several international meetings were held in 1978, including an expert committee of the World Health Organisation in Geneva on the newer sexually transmitted diseases. Other conferences took place in Zurich, Vienna, New Delhi, Paris, Perth (Western Australia), and San Francisco. British physicians took part and contributed papers at all these meetings.

The DHSS continues to make available a sum of money for workers in the field to visit centres overseas to learn new techniques and to study special aspects of the subject. These grants have been of great value in enabling British doctors, nurses, contact-tracers, and social workers to study disease control overseas.

Health education about the sexually transmitted diseases requires further development in the United Kingdom. Because of the continuing high prevalence of most of these diseases, further developments in this aspect of the subject are desirable, both at local and national levels.

Reference

1. Department of Health and Social Security. Sexually transmitted diseases. *On the State of the Public Health: The Annual Report of the Chief Medical Officer of the Department of Health and Social Security for the Year 1978.* London: HMSO. 1979:60-65.

Br J Vener Dis 1980;56:182.

Annual report based on the Secretary's report to the Annual General Meeting on 26 October 1979

Medical Society for the Study of Venereal Diseases

During the past year 69 new members were elected, of whom 31 are resident in the United Kingdom. Four members died and eight resigned. At present the membership stands at 696.

Five ordinary meetings were held during the session. The excellent standard of attendance that was attained during the previous year was well maintained and there was lively discussion at each meeting with many members taking part.

The spring meeting was held in Lübeck. It was very successful and our thanks are extended to Professor D Petzoldt, the local organiser, for his splendid work. Our congratulations are also offered to him on his appointment to the chair at Heidelberg, which is particularly gratifying as it is the first time that such an appointment in West Germany has gone to one whose interests are venereological rather than dermatological.

The Council of the Society under the chairmanship of the President, Dr E M C Dunlop, held five meetings, all of which were well attended.

At the October meeting, Dr R D Catterall reported on the European Union of Medical Specialties. He said discussions were continuing, but no progress had been made, over the difficulty of the specialty of dermatovenereology.

During subsequent meetings Dr C B S Schofield announced that in future a senior registrar would be on the Specialist Advisory Committee, serving for 12 months. Concern was expressed about the imbalance between the number of senior registrars and the number of available consultant posts. It was noted that consultant posts would become progressively more difficult to obtain.

Following Major Mitchell's retirement, the Council agreed that the Treasurer should send him a Harrod's gift token for £50 in appreciation of all the help he had given during his term of office.

The *British Journal of Venereal Diseases* continues to flourish under the guidance of Dr R N Thin and his Editorial Committee, who merit our congratulations on maintaining the high standard to which we have become accustomed. At the January meeting, Dr Thin announced that Dr G Csonka and Dr A McMillan had agreed to serve on the Editorial Committee and that Dr A E Wilkinson had agreed to act as the next Editor on a temporary basis. He said that the Editorial Committee would recommend two names to Council every year. The President expressed thanks to the retiring members.

At the last meeting in April, following the voting on the timing of meetings, it was agreed that in future ordinary general meetings would start at 7 pm.

At this meeting it was also agreed that there should be a joint meeting with the proctologists during the 1979/80 session.

All in all I am glad to report that the Society continues to flourish and indeed it would seem fair to say that it goes from strength to strength, but we must remember that its future health depends on how much each one of us puts into it.

Finally, I should like to thank all those who have helped me during the past year, especially your President, Dr Eric Dunlop, and the Council, who have been generous in their support.

J BARROW
Honorary Secretary

Br J Vener Dis 1980;56:183-4.

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

Chlamydia culture service

We read with interest the paper by J R Willcox *et al*[1] advocating a routine chlamydial isolation service. They found that almost 40% of *Chlamydia trachomatis*-positive female patients had no history of contact with a patient with urethritis and therefore would not have received antichlamydial therapy. Kinghorn and Waugh[2] have recently published similar data. The results of previous series,[3, 4] however, have suggested that chlamydia are rarely found in the absence of a history of contact with either nongonococcal urethritis (NGU) or gonorrhoea.

We are conducting a study of unselected female patients attending two venereal disease clinics. So far, *C trachomatis* has been isolated from 50 (21%) of 241 patients, a figure compatible with those of other studies.[1-4] Of these 50 patients, 40 had a definite history of contact with a man suffering from NGU, gonorrhoea, or an unspecified urethritis, and another two women had an infection with *Neisseria gonorrhoeae* and can therefore be presumed to have had a contact in the above categories. Only eight (16%) chlamydia-positive patients therefore had no contact history at all. If our study is analysed in terms of diagnostic categories, 25 (31%) of 81 contacts of NGU and 15 (33%) of 44 contacts of gonorrhoea were chlamydia-positive but only eight (7%) of 116 patients with no contact history were chlamydia-postive.

In the absence of a chlamydial isolation service, at present only those patients who have a history of contact with NGU are considered for antichlamydial therapy. It has, however, been suggested[5] that women with gonorrhoea or with a history of contact with gonorrhoea should receive a treatment regimen which is effective against chlamydia in the absence of any simple screening test for the diagnosis of postgonococcal cervicitis. Our results suggest that if all women with a history of contact with NGU or gonorrhoea or with an infection with *N gonorrhoeae* had received antichlamydial therapy, only a small proportion of chlamydia-positive patients would have remained untreated.

There thus seem to be two possibilities in dealing with the reservoir of female chlamydial infection in the community: either a routine chlamydial isolation service analogous to that for *N gonorrhoeae* is set up for every clinic or therapeutic regimens are designed which will eradicate a large proportion of chlamydial infections. Although others[1, 2] may feel that their results indicate that laboratory assistance is essential for treatment, our results lead us to believe that institution of such therapeutic regimens would be a justifiable course of action. We do not believe that it would be cost-effective with present chlamydial isolation techniques to set up a routine service and we would favour the restriction of chlamydial isolation to epidemiological and other studies.

We would like to thank Drs G Csonka and J Nabarro for access to patients under their care.

Yours faithfully,
D Taylor-Robinson
P E Munday

Clinical Research Centre,
Watford Road,
Harrow,
Middlesex HA1 3UJ

References

1. Willcox JR, Fisk PG, Barrow J, Barlow D. The need for a chlamydia culture service. *Br J Vener Dis* 1979;55:281-3.
2. Kinghorn GR, Waugh MA. (Letter) *Br Med J* 1979;ii:44.
3. Oriel JD, Powis PA, Reeve P, Miller A, Nicol CS. Chlamydial infections of the cervix. *Br J Vener Dis* 1974;50:11-16.
4. Hilton AL, Richmond SJ, Milne JD, Hindley F, Clarke SKR. *Chlamydia A* in the female genital tract. *Br J Vener Dis* 1974;50:1-10.
5. Richmond SJ, Oriel JD. Recognition and management of genital chlamydial infection. *Br Med J* 1978;ii:480-3.

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

Effect of probenecid on amoxycillin

In common with many other doctors, I find the mathematics of clinical pharmacology difficult and would be grateful for some clarification of the information in the paper by Barbhaiya *et al*[1] about the effect of probenecid on amoxycillin. They say that the AUC was taken as a measure of relative absorption of amoxycillin in the presence and absence of probenecid, and they found it to be increased with probenecid. This would seem to imply that amoxycillin is better absorbed when probenecid is present, although it is not obvious why the AUC differences could not be simply due to the change in excretion. Or is "relative absorption" a technical term meaning "absorption relative to metabolism/excretion"?

It is well known that probenecid delays the excretion of penicillins, but while the prolonged concentrations obtained can be accounted for by this alone, the mechanism causing increased concentrations is less clear. It has been shown that delayed excretion is insufficient to account for the extent of the increased serum concentrations, but the suggestion is that probenecid interferes with the distribution of the antibiotics in the body rather than increasing their absorption.[2-4] The implication of this is that although impressive serum concentrations are obtained, these are achieved at the expense of antibiotic in the tissues.

In view of the clinical results, this is obviously not a critical factor in the single-dose therapy of urethral gonorrhoea, but it could be important in other situations such as the treatment of gonococcal salpingitis. The relationship between serum and tissue concentrations of amoxycillin seems to be similar to that of ampicillin,[5] and it has been shown that the concentration of ampicillin in the Fallopian tubes is only one-quarter of that in the serum.[6] It must be at least a possibility that the addition of probenecid would further lower the concentrations in the Fallopian tubes, exactly the opposite of the effect that might have been expected.

Yours faithfully,
Kevin Woodcock

Special Clinic,
King Edward VII Hospital,
Windsor

References

1. Barbhaiya R, Thin RN, Turner P, Wadsworth J. Clinical pharmacological studies of amoxycillin: effect of probenecid. *Br J Vener Dis* 1979;55:211-3.

2. Gibaldi M, Schwartz MA. Apparent effect of probenecid on the distribution of penicillin. *Clin Pharmacol Ther* 1968;**9**:345-9.
3. Gibaldi M, Davidson, D, Plant ME, Schwartz MA. Modification of penicillin distribution and elimination by probenecid. *Int J Clin Pharmacol* 1970;**3**:182-9.
4. Ziv G, Sulman FG. Effects of probenecid on the distribution, elimination, and passage into milk of penicillin, ampicillin and cloxacillin. *Arch Int Pharmacodyn Ther* 1974;**207**:373-82.
5. Tan JS, Bannister T, Pari JP. Level of amoxycillin in human serum and intestitial fluid. *J Inf Dis* 1974;**129** suppl June:146.
6. Elder MG, Bywater MJ, Reeves DS. Pelvic tissue and serum concentrations of various antibiotics. *Br J Obstet Gynaecol* 1977:**84**;887-93.

***A copy of this letter was sent to the authors, whose reply is printed below. ED, *BJVD.*

Sir,

We are grateful to Dr Woodcock for drawing attention to the important question of a possible effect of probenecid on the apparent volume of distribution of amoxycillin. Although he rightly points out that Gibaldi and co-workers had suggested that such an effect occurs, we would refer him to a later paper by Jusko and Gibaldi,[1] in which they demonstrated that, while alteration of elimination produces a change in the degree of equilibration of a drug between the central and peripheral compartments which affects certain apparent "volume of distribution" parameters, no change in distribution mechanisms or space necessarily occurs. After reanalysing their original data, they concluded that "the distribution rates and space of penicillin do not appear to be significantly altered by probenecid...".

P Turner
R Barbhaiya
R N Thin

Departments of Clinical Pharmacology
and Genital Medicine,
St Bartholomew's Hospital Medical
College,
London EC1A 7BE

Reference

1. Jusko WJ, Gibaldi M. Effects of change in elimination on various parameters of the two-compartment open model. *J Pharm Sci* 1972;**61**:1270-3.

TO THE EDITOR, *British Journal of Venereal Diseases*

Sir,

Piperacillin

I refer to the paper by Waterworth *et al*,[1] which reported on the antigonococcal activity of a number of agents including piperacillin.

In the last paragraph of this article, it is stated that piperacillin is a carbenicillin. So as to avoid any misunderstanding, I should point out that piperacillin is in fact a dioxopiperazinylacetyl derivative of ampicillin and its structure and activity are sufficiently different from carbenicillin to make the statement inaccurate.

Yours faithfully,
Maryanne Roach

Lederle Laboratories,
Fareham Road,
Gosport,
Hampshire PO13 0AS

Reference

1. Waterworth PM, Oriel JD, Ridgway GL, Subramanian S. Single-dose minocycline in the treatment of gonococcal urethritis. *Br J Vener Dis* 1979;**55**:343-7.

***This letter has been shown to Dr Waterworth, who agrees that a more accurate description would have been "carbenicillin-like." ED, *BJVD.*

Br J Vener Dis 1980;**56**:185.

Book reviews

Sexually Transmitted Diseases: The Facts. David Barlow, 1979. Pp 140, 8 figs. Oxford University Press, Oxford (£4.50).

This book on sexually transmitted diseases will be of great interest to a wide range of readers, including health educators and those in other health professions. It provides current factual knowledge on the main venereal diseases and on a variety of infections and infestations transmitted sexually.

Several sections of the book have special appeal. In the chapter on "Sexual anatomy and function" there are excellent illustrations with a readable text, including an important section on variations in sexual behaviour. In this and in other chapters dealing with specific infections, reference is made to homosexuality and the prevalence of sexually transmitted diseases in this group.

The chapter on "History and development of a service" provides educationalists with an excellent basis for inclusion in any programme on this particular aspect of human relationships. This section concludes with the evolution of clinical services in this country, which are recognised to be the best in the world. The author helpfully provides a complete list of clinics in the United Kingdom at the very end of the book.

The section on tropical diseases is an important addition to any book on sexually transmitted diseases, as it contains valuable information on the increasing minority of ethnic grouping in urban areas of this country and on the Continent.

Chapters on each of the infections contain a wealth of information on signs, symptoms, treatment, and incidence of the respective infections. The lay reader may well need to refer to the glossary of clinical terms provided by the author at the back of the book.

Details are given on the location of clinics, their staffing, confidentiality, and treatment, which, I am sure, will help to overcome the stigma of attending a hospital for treatment of diseases of a sexual nature. This will be useful for young people, who are considered to be a high-risk group.

The author finally discusses control and various factors which he considers will help to curb the worldwide increase in these diseases.

The amusing cartoons by Dickinson provide a good counter-balance to the superb medical illustrations in the book.

I consider that this book will be a welcome addition to libraries in schools, colleges for further education, teaching hospitals, as well as in health education units, health centres, and medical group practices. It may well be useful for copies to be sent to the Gay Liberation Movement and to the Campaign for Homosexual Equality.

G Peters

Boswell's Clap and Other Essays: Medical Analyses of Literary Men's Afflictions. William B Ober, 1979. Pp 291, 10 figs. Southern Illinois University Press, Carbondale and Edwardsville, Feffer and Simons, London and Amsterdam (US$17.50).

It is a pity that this title has been chosen for such a recondite collection of superb essays, attracting the voyeur in many a physician browsing in a medical bookshop. Dr W Ober, a histopathologist, has been recognised for many years as a leading American medical historian coming from the school of scholar-philosophers found on the eastern seaboard. He takes 10 literary figures or groups; Boswell, Swinbourne, D H Lawrence, John Keats and George Crabbe, Collins, Cowper and Smart, Chekhov, William Carlos Williams, the Earl of Rochester, Thomas Shadwell, and Socrates, and analyses aspects of their lives, using the skills of his specialty. In this process he makes some remarkable, but probably correct, diagnoses, often backed by psychopathological arguments. His groundwork cannot be faulted, taking into consideration literary and historical published work as well as primary sources. Venereologists will find not only the title essay illuminating but also a great deal of interest in pyschosexual pathology in at least half the other essays, notably those on Swinbourne, Keats, D H Lawrence, and the Earl of Rochester.

Ober details 19 instances of urethritis, mostly probably gonococcal in origin in Boswell's life between the age of 19 and 50, an attack of crab lice, and one of genital sores, probably not syphilitic. What seems most interesting are the psychological reasons for Boswell's repeated infections, despite—in latter years—a constant wife and his frequent visits to a succession of medical men. Not only did he consult surgeons no longer remembered but also doctors who are celebrated today: Morgagni in Padua, Pott in London, as well as Pringle and Gregory in Edinburgh.

An early example of contact-tracing is when Boswell paid for the treatment at St Thomas's Hospital of Betsey Smith, a prostitute from whom he contracted gonorrhoea in 1785. It is also worth remembering in these days of easy treatment the fate of Boswell, dying at 55 of uraemia, the result of acute and chronic urinary tract infection secondary to postgonococcal urethral stricture.

Michael Waugh

Correction

In the paper by Dr K C Nayyar *et al* on the "Rising incidence of chancroid in Rotterdam" (1979;55:439-41) the figure in the last line of the fourth paragraph of the section on patients and methods should have been 7·4% and not 77%.

Br J Vener Dis 1980;**56**:186-191

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
  (Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea
  (Clinical; microbiology; therapy)
Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases
Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (clinical and therapy)

Syphilis and homosexuality in adolescents
LR JAFFE AND JE MORGENTHAU (Adolescent Health Centre, New York, USA). *J Pediatr* 1979;**95**:1062-4.

Although venereologists are aware of the insidiousness of the onset of syphilis, other physicians nowadays see few cases of syphilis and often forget its manifestations. In the British Isles, paediatricians would still not be dealing with the cases mentioned in this study.

Case reports are given of a 17-year-old boy, misdiagnosed at first as having pityriasis rosea and later found to have secondary syphilis, and a 16-year-old boy found to have positive treponemal serological tests; both presented to paediatricians. They had contracted the disease through homosexual contact. Contact tracing was initiated after diagnosis.

M A Waugh

Pachymeningitis cervicalis hypertrophica syphilitica
N AGDAL, HK HAGDRUP, AND GL WANTZIN (University of Copenhagen, Denmark). *Acta Derm Venereol (Stockh)* 1980;**60**:184-6.

Syphilis (serology and biological false-positive phenomenon)

Syphilis antibodies in the cerebrospinal fluid and their diagnostic significance
HJ HAGEDORN (University of Düsseldorf, West Germany). *Dtsch Med Wochenschr* 1980;**105**:155-60.

Samples of serum and cerebrospinal fluid (CSF) of 45 patients with confirmed syphilis were tested by the TPHA titre, specific IgG, and albumin to determine the ratio in the two specimens and evaluate the possibility of local production of specific antibodies in the central nervous system (CNS). In 10 of 11 patients with active neurosyphilis local production of antibodies in the CNS was demonstrated. In 16 patients with possible active neurosyphilis, seven specimens of CSF showed local antibody production and in none of 18 patients without evidence of neurosyphilis were local CNS antibodies found. It is concluded that the presence of immunoglobulins and positive TPHA test results at appropriate titres suggesting local production of CNS antibodies are a valuable diagnostic approach in the diagnosis of active neurosyphilis.

G W Csonka

Comparison of serum and plasma specimens for syphilis serology using the reagin screen test
JD DYCKMAN AND RD WENDE (Houston City Health Dept Laboratory, Texas, USA). *J Clin Microbiol* 1980;**11**:16-8.

The Wasserman, Kline and VDRL reactions in routine syphilis serodiagnosis
E BERNTSSON AND P LARSSON (University of Goteberg, Sweden). *Acta Derm Venereol (Stockh)* 1980;**60**:71-2.

Syphilis (pathology and experimental)

Surface-associated host proteins on virulent *Treponema pallidum*
JF ALDERETE AND JB BASEMAN (University of North Carolina, USA). *Infect Immun* 1979;**26**:1048-56.

Characterization of lymphocyte responsiveness in early experimental syphilis. I In-vitro response to mitogens and *Treponema pallidum* antigens
SA LUKEHART, SA BAKER-ZANDER, AND S SELL (University of California, USA). *J Immunol* 1979;**124**:461-7.

Testicular cultivation of *Treponema pallidum* (Nichols strain) facilitated by sustained release steroid administration
BD BRAUSE, S QUALLS, AND RB ROBERTS (Cornell University, New York, USA). *J Clin Microbiol* 1979;**10**:937-9.

Histopathology of secondary syphilis
M PATEROU, N STAVRIANEAS, J CIVATTE, AND J CAPETANAKIS (Hôpital St Louis, Paris, France). *Ann Dermatol Venereol* 1979;**106**:923-7.

Syphilitic lymphadenitis: immunofluorescent identification of spirochetes from imprints
YJ CHOI AND L REINER (Bronx Lebanon Hospital Centre, New York, USA). *Am J Surg Pathol* 1979;**3**:553-6.

Cell-mediated immunity in *Treponema pallidum* infected rabbits; in-vitro response of splenic and lymph node lymphocytes to mitogens and specific antigens

SM MARET, JB BASEMAN, AND JD FOLDS (University of North Carolina, USA). *Clin Exp Immunol* 1980;**39**:38-43.

Les complexes immuns circulants dans la syphilis primo-secondaire et sérologique

F PIETTE, P WATTRE, J-P DESSAINT, P DEVEMY, AND H BERGOEND (Hôpital Regional, Lille Cedex, France). *Ann Dermatol Venereol* 1979;**106**:967-72.

Some clinical features of syphilis suggest that immune complexes may be a pathogenetic factor in the syphilitic lesions. Recently, circulating immune complexes have been reported in six patients with secondary syphilis by Sølling *et al.*

In our study, the presence of circulating immune complexes was investigated in 42 patients with syphilis (primary, secondary, latent) by the method of C1q binding test. Elevated C1q binding activity was demonstrated in two-thirds of the patients with primary and secondary syphilis, with a significant difference between this group and the controls. Only two of the 21 patients with latent syphilis showed elevated C1q binding activity. Circulating immune complexes, often at moderate rates, appear very early and decrease rapidly during treatment.

It was not possible to demonstrate a decline in serum complement in association with elevated C1q binding activity.

During five Jarisch-Herxheimer reactions, there was no increase in circulating immune complexes compared with pretreatment values: this suggests that circulating immune complexes have no essential importance in this reaction.

The characterisation of the components of these circulating immune complexes by the previously described "radioimmunoprecipitation PEG assay" (RIPEGA) will enable us to state their specificity and to conceive their potential responsibility in some lesions of secondary syphilis, such as nephrotic syndrome.

Authors' summary

Rates of clearance of virulent *Treponema pallidum* (Nichols) from the blood stream of normal *Mycobacterium bovis* BCG-treated and syphilitic rabbits

S GRAVES (Monash University, Melbourne, Australia). *Infect Immun* 1980;**27**:264-7.

This paper gives the results of experiments to determine whether BCG-treated rabbits cleared *Treponema pallidum* from their bloodstream more rapidly than rabbits in which immunity to *T pallidum* had been produced either actively or passively.

Five groups of rabbits were used: (A) six normal rabbits to provide a baseline clearance rate; (B) three rabbits inoculated with 1 mg *Mycobacterium bovis* (BCG) between four and six weeks earlier (these animals gave positive tuberculin test results); (C) three rabbits treated with immune serum prepared from other rabbits previously infected with *T pallidum* between six and 24 months earlier (the serum was given by daily intravenous injections of 10 ml commencing six days previously and with a final injection two hours before the challenge with *T pallidum*); (D) three rabbits treated with immune serum and BCG as in groups B and C; (E) three rabbits infected with *T pallidum* 13 months previously with present serology RPR + TPHA + 1/5120.

Intravenous injections with suspensions of freshly minced syphilitic orchitic testes containing a mean total of $1 \cdot 3 \times 10^8$ *T pallidum* in an unspecified quantity of an anaerobic maintenance medium were given in an unspecified vein. Thereafter 0·5 ml amounts of blood were withdrawn from a marginal ear vein and 0·1 ml aliquots of whole blood injected intradermally in four sites on the shaved back of indicator rabbits. No anticoagulant was used and the injections were performed within two minutes. The indicator rabbits were kept shaved in a room temperature of 18°C. They were not immunosuppressed.

A clearance time was taken as the time when the circulating concentration of treponemes would establish lesions in only two of the four sites on indicator rabbits. These were: group A, 90 mins, group B, 90 mins, group C, 16 mins, group D, 54 mins, and group E, 20 mins. Complete clearance times were group A and B, > 8 hours, group C, 32-60 mins, group D, 1-2 hours, and group E, 2-4 hours. From these results it is inferred that the activation of the fixed macrophages in the reticuloendothelial system by BCG does not enhance the clearance of *T pallidum*, synergy with passive humoral immunity does not take place, and circulating humoral factors—probably antibodies to *T pallidum*—play a role in immunity to reinfection in syphilis.

G D Morrison

Experimental syphilis and serological examination for treponematosis in hares

I HORVATH, F KEMENES, L MOLNAR, A SZEKY, AND I RACZ (Allegheny Centre, Pittsburgh, USA). *Infect Immun* 1980;**27**:231-4.

Concanavalin A-mediated affinity film for *Treponema pallidum*

JB BASEMAN, Z ZACHAR, AND NS HAYES (University of North Carolina, USA). *Infect Immun* 1980;**27**:260-3.

Experimental syphilis in the rabbit: passive transfer of immunity with immunoglobulin G from immune serum

RG TITUS AND RS WEISER (National Jewish Hospital, Denver, USA). *J Infect Dis* 1980;**140**:904-13.

Genetic relationship between *Treponema pallidum* and *Treponema pertenue*, two non-cultivable human pathogens

RM MIAO AND AH FIELDSTED (Menlo Park, California, USA). *J Bacteriol* 1980; **141**:427-9.

Gonorrhoea (clinical)

Characteristics of defaulters in treatment for infection with *Neisseria gonorrhoeae*

GL GOODHART, M KRAMER, AND AA ZAIDI (Centre for Disease Control, Atlanta, USA). *J Infect Dis* 1979;**140**:649-51.

Orbital cellulitis due to *Neisseria gonorrhoeae* in an enucleated socket

JJ FRAZIER, J MILLER, AND LK PICKERING (University of Texas, USA). *Arch Ophthalmol* 1979;**63**:2345.

Gonorrhoea (microbiology)

The in-vitro and in-vivo effects of a surgical lubricant on the recovery of gonococci from the endocervical canal

MR SPENCE, M BROCKMAN, M SWEITZER, J SMITH, AND T MOISSON-PULLIAM (Johns Hopkins University, Baltimore, USA). *Obstet Gynecol* 1979;**54**:746-9.

Disseminated gonococcal infection in mice.

LB CORBEIL, AC WUNDERLICH, RR CORBEIL, JA McCUTCHAN, JI ITO, AND AI BRAUDE (University of California, USA). *Infect Immun* 1979;**26**:984-90.

Comparison of two selective media in the cultural diagnosis of gonorrhea
PL SVARVA AND JA MAELAND (University of Trondheim, Norway). *Acta Pathol Microbiol Scand* 1979;**87:**391-2.

Lectins in diagnostic microbiology: use of wheat germ agglutinin for laboratory identification of *Neisseria gonorrhoeae*
RL SCHAEFER, KF KELLER, AND RJ DOYLE (University of Louisville, USA). *J Clin Microbiol* 1979;**10:**669-72.

A lectin slide agglutination test has been developed for the confirmatory identification of *Neisseria gonorrhoeae*. With wheat germ lectin as an agglutinin, 164 of 165 clinical isolates of *N gonorrhoeae* gave a 3 to 4+ reaction within six to eight mins. Four gonococcal isolates, even though giving negative results by the fluorescent-antibody method, gave strong positive reactions with the wheat germ lectin. Among 23 isolates of *Neisseria meningitidis* tested, which included representatives of serogroups A, B, C, D, X, Y, and Z, only one strain in group X gave a false-positive reaction. The nonpathogenic species of *Neisseria*, as well as *Branhamella catarrhalis*, all showed negative reactions with the wheat germ agglutinin. The novel method provides a simple, rapid, and inexpensive means for the laboratory diagnosis of gonorrhoea and obviates the need for performing second-stage sugar fermentation studies or using the more expensive fluorescent-antibody techniques.

Authors' summary

The role of natural IgG and complement in the phagocytosis of type 4 *Neisseria gonorrhoeae* by human polymorphonuclear leukocytes.
NL SCHILLER, GL FRIEDMAN, AND RB ROBERTS (Cornell University Medical College, New York, USA).

The role of human serum components in the phagocytosis of logarithmic-phase type 4 *Neisseria gonorrhoeae* by human polymorphonuclear leucocytes was investigated. The requirement of fresh normal human serum (FHS) for optimal phagocytosis and the fixation of human immunoglobulin (IgG) and complement (C3) to the gonococcal cell surface suggested that both serum factors participate in the phagocytosis of these organisms. The percentage of neutrophils containing ingested organisms was directly proportional to the concentration of IgG purified from FHS. Absorption studies suggested that this natural IgG binds to a trypsin-sensitive surface protein on type 4 gonococci and crossreacts with stationary-phase type 2 *N gonorrhoeae*, group C *Neisseria meningitidis*, and *Branhamella catarrhalis*, but not with logarithmic-phase type 2 gonococci or other *Neisseria* species. Although complement alone did not promote phagocytosis, it enhanced IgG-mediated ingestion. Studies using C2-deficient serum or serum chelators indicated that the alternative complement pathway participates in this interaction.

Authors' summary

Deoxyribonucleic acid repair capacities of *Neisseria gonorrhoeae:* absence of photoreactivation
LA CAMPBELL AND RE YASBIN (Pennsylvania State University, USA). *J Bacteriol* 1980;**140:**1100-1.

Gonorrhea screening: comparison of three techniques
AA LUCIANO AND L GRUBIN (University of Connecticut, USA). *JAMA* 1980;**243:** 680-1.

Biological properties of two distinct pilus types produced by isogenic variants of *Neisseria gonorrhoeae* pa
PR LAMBDEN, JN ROBERTSON, AND PJ WATT (University of Southampton, UK). *J Bacteriol* 1980;**141:**393-6.

High molecular weight antigenic protein complex in the outer membrane of *Neisseria gonorrhoeae*
WJ NEWHALL, CE WILDE, WD SAYER, AND RA HAAK (University of Indiana, USA). *Infect Immun* 1980;**27:**475-82.

Evaluation of the Phadebact gonococcus test, a coagglutination procedure for confirmation of *Neisseria gonorrhoeae*
JS LEWIS AND JE MARTIN (Centre for Disease Control, Atlanta, USA). *J Clin Microbiol* 1980;**11:**153-6.

Gonorrhoea (therapy)

Treatment of gonorrhoea caused by β-lactamase-producing strain of *Neisseria gonorrhoeae* with cefotaxime (letter)
RCB SLACK, JB BITTINER, AND R FINCH (University of Nottingham, UK). *Lancet* 1980;i:431-2.

The authors describe the use of the cephalosporin, cefotaxime, in a 22-year-old woman who had been infected by her boyfriend who had acquired the disease in the Far East. The infecting strain of the gonococcus was identified as a β-lactamase producer. Although the organism was sensitive in vitro to erythromycin, treatment with this drug, in an oral dose of 1 g daily for seven days, was ineffective. After treatment with a single intramuscular 2-g dose of cefotaxime, microbiological examination failed to demonstrate infection.

A McMillan

Pharmacological and in-vitro evaluation of cyclacillin: assessment as potential single-dose therapy for treatment of *Neisseria gonorrhoeae* infection
KF WAGNER, AD BLAIR, GW COUNTS, AND KK HOLMES (University of Washington, Seattle, USA). *Antimicrob Agents Chemother* 1980;**17:**89-91.

Non-specific genital infection

Persistent urethral leucocytosis and asymptomatic chlamydial urethritis
SL SWARTZ AND SJ KRAUS (Centre for Disease Control, Atlanta, USA). *J Infect Dis* 1979;**140:**614-7.

This paper follows up a previous report from these workers, concerning a definition of asymptomatic nongonococcal urethritis (NGU) based on the number of polymorphonuclear leucocytes (PMN) in the urethral specimen. Fifty-six sexually active men, without symptomatic urethritis or dysuria, were examined for PMN in a urethral smear. Those with >4 PMN/high-power field (hpf) were the controls. Patients were assessed at one week, and each group further sub-divided into those with >4 PMN/hpf and those with <4 PMN/hpf. Fifty-five per cent of the asymptomatic group converted to <4 PMN/hpf after one week. Of the 45% still with 4 PMN/hpf, six of the 13 patients yielded *Chlamydia trachomatis.* Within the asymptomatic and control groups, the isolation rates of *C trachomatis* were seven of 29 men and two of 27 men respectively; these figures approach significance. There was no correlation between the presence of *Ureaplasma urealyticum* and the asymptomatic or control groups.

It is concluded that persistence of >4 PMN/hpf in the urethral smear of men with asymptomatic NGU may be a useful indicator of infection with *C trachomatis* in clinics where isolation facilities for this organism are not readily available.

One criticism of the paper which could affect the results is that we are not told the time between last micturition and examination of urethral smears.

G L Ridgway

A method for the preparation of a chlamydia-group specific antigen on HeLa—229 cells infected with a strain of *Chlamydia trachomatis* for use in the complement fixation test

R COLIMON, F FERCHAL, AND Y PEROL (Hôpital St Luzare, Paris, France). *Ann Microbiol* 1979; **130**: 313-21.

Chlorhexidine as an effective agent against *Chlamydia trachomatis* in vitro and in vivo

IT NISBET, DM GRAHAM, PE SPICER, AND GJ TIBBS (University of Melbourne, Australia). *Antimicrob Agents Chemother* 1979; **16**: 855-60.

Isolation of *Chlamydia trachomatis* from the lower respiratory tract of adults

KJ TACK, PK PETERSON, FL RASP, M O'LEARY, D HANTO, RL SIMMONS, AND LD SABATH (University of Minnesota, USA). *Lancet* 1980; i: 116-20.

Lower respiratory tract specimens from 46 adult patients with pulmonary infections were cultured for *Chlamydia trachomatis*. Isolation was achieved in six patients with conditions varying from acute bronchitis to severe diffuse interstitial pneumonia; these cases are discussed in detail. Four patients were immunosuppressed, three after renal transplantation, and one had acute lymphatic leukaemia. Cytomegalovirus was also isolated from those receiving renal allografts. Two immunosuppressed patients died; two improved rapidly with doxycycline, one with erythromycin, and the other slowly with penicillin.

This is the first report of isolation of *C trachomatis* from the lower respiratory tract of adults. The pathogenesis is discussed briefly with particular reference to morbidity and mortality in the immunosuppressed patient.

R S Pattman

Chlamydial endocarditis (editorial)

Lancet 1980; i: 132.

Significance of chlamydial genital infection in male infertility

V NIKKANEN, P TERHO, R PUNNONEN, AND O MEURMAN (University of Turku, Finland). *Arch Androl* 1980; **4**: 57-62.

Techniques for culturing and determining antimicrobial susceptibility of *Chlamydia trachomatis*

TR ROTA (Massachusetts General Hospital, Boston, USA). *Arch Androl* 1980; **4**: 63-8.

The role of *Chlamydia trachomatis* in genital tract and associated diseases

D TAYLOR-ROBINSON AND BJ THOMAS (Clinical Research Centre, Harrow, UK). *J Clin Pathol* 1980; **33**: 205-33.

Infection of untreated primary human amnion monolayers with *Chlamydia trachomatis*

HR HARRISON AND RT RIGGIN (University of Arizona, USA). *J Infect Dis* 1980; **140**: 968-71.

Experimental infection of the genital tract of female Grivet monkeys by *Mycoplasma hominis*: effects of different routes of infection

BR MØLLER AND EA FREUNDT (Aarhus University, Denmark). *Infect Immun* 1979; **26**: 1123-8.

Enhancement of *Ureaplasma urealyticum* growth on a differential agar medium (A 7 B) by a polyamine, putrescine

MC SHEPARD AND RS COMBS (US Navy). *J Clin Microbiol* 1979; **10**: 931-3.

Reiter's Disease

Nail involvement in Reiter's syndrome

JWE DIGKSTRA (Groningen, the Netherlands). *Br J Dermatol* 1980; **102**: 480-2.

Trichomoniasis

Serodiagnosis of *Trichomonas vaginalis* infection by the indirect fluorescent antibody test

PR MASON (Salisbury, Zimbabwe). *J Clin Pathol* 1979; **32**: 1211-5.

In this study the presence of antibodies to *Trichomonas vaginalis* in patients with asymptomatic trichomonal infections was investigated by using the indirect fluorescent antibody (IFA) test.

Cultures of *T vaginalis* from each of seven patients attending an antenatal clinic (Sp-antigen) as well as a mixture of cultures from all seven patients (co-antigen) were used to prepare the antigen slides. Sera were obtained from 200 antenatal patients and 30 prepubescent girls. Standard IFA procedures were used.

Of the sera from the antenatal patients, 104 (52%) gave positive results with co-antigen. Among the 52 patients with confirmed trichomoniasis in this group, 90% had antibodies at a concentration of 1/4, but among those in whom trichomonads were not detected only 17% had positive results at the same dilution whereas 64% had no demonstrable antibody. Only one of the sera from the children gave a positive reaction.

A comparison of tests using sp-antigen and those using co-antigen showed a good correlation. It appeared that IgG rather than IgM was the class of antibody concerned.

The results of the study indicated that the IFA test was reasonably reliable. Its value lay in the diagnosis of male patients as well as of women with chronic low-grade infection, since in both these groups the demonstration of trichomonads is difficult.

C S Ratnatunga

Candidosis

Inhibition of candidacidal activity of human neutrophil leukocytes by aminoglycoside antibiotics

FA FERRARI, A PAGANI, M MARCONI, R STEFANONI AND AG SICCARDI (University of Pavia, Italy). *Antimicrob Agents Chemother* 1980; **17**: 87-8.

Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells

RD KING, JC LEE, AND AL MORRIS (University of Texas, USA). *Infect Immun* 1980; **27**: 667-74.

Genital herpes

Transient neurogenic bladder in genital herpes

TW CHANG (New England Medical Centre Hospital, Boston, USA). *J Infect* 1979; **1:** 375-8.

Acyclovir for suspected systemic herpes infections (letter)

EL TEARE AND MR CLEMENTS (Westminster Hospital, London, UK). *Lancet* 1980; i: 42.

Herpes simplex encephalitis in pregnancy

RL KING (North Hills Passavant Hospital, Pittsburgh, USA). *Am J Obstet Gynecol* 1979; **135:** 1114-5.

Storage and transport of cultures for herpes simplex virus type 2

AS YEAGER, JE MORRIS, AND CG PROBER (University School of Medicine, Stanford, California, USA). *Am J Clin Pathol* 1979; **72:** 977-9.

Levamisole plus indomethacin in the treatment of herpes simplex

E HECHANDEX-PEREZ (University of El Salvador). *Dermatologica* 1980; **160:** 118-21.

Different susceptibilities of skin to type 1 and type 2 herpes simplex viruses in newborn rabbits

JO OH AND P MINASI (University of California, USA). *Infect Immun* 1980; **27:** 168-74.

Other sexually transmitted diseases

Aminopeptidase activity in *Corynebacterium vaginale*

M PERDIZ, P BOURLIOUX, J FOURNIAT, AND A GERMAN (Centre Etude Pharmaceutique, Chatenay, Malabry, France). *Ann Microbiol* 1979; **130:** 375-8.

Corynebacterium vaginale and vaginitis: a controlled trial of treatment

MJ BALSDON, GE TAYLOR, L PEAD, AND R MASKELL (St Mary's Hospital, Portsmouth, UK). *Lancet* 1980; i: 501-3.

The clinical and microscopical diagnosis of *Corynebacterium vaginale* vaginitis was compared with laboratory culture, and double-blind treatment with metronidazole, oxytetracycline, and placebo assessed.

Vaginal discharge was examined for infection with *C vaginale* by Gram stain (Gram-variable coccobacilli and clue cells), wet film (clumps of coccobacilli and clue cells), vaginal pH, and the amine test (10% potassium hydroxide added to the wet-mount preparation and examined for a "fishy" odour). Further vaginal samples were cultured on Columbia chocolate agar for *C vaginale.* Patients with concomitant infection were excluded from the study as well as those with an intrauterine contraceptive device, those who had taken recent antimicrobial medication, contacts of non-specific urethritis, and those with a mucopurulent cervical discharge.

Thirty such women with a malodorous vaginal discharge were selected by the described microscopical findings and treated by the randomised double-blind method with metronidazole (400 mg twice daily), oxytetracycline (500 mg twice daily), or two placebo tablets twice daily, all for one week. Microscopy and microbiological investigations were repeated after 10 days and four weeks, and cure was accepted in the absence of an abnormal vaginal discharge and normal microscopy. Treatment failures were given one week's course of metronidazole (400 mg twice daily).

Laboratory culture confirmed the diagnosis in all but two cases initially and in 10 of 13 patients at follow up. *Corynebacterium vaginale* was found nine times when it was not suspected clinically. Vaginal pH was above 5·2 in all cases where *C vaginale* was found and was between 4·2 and 4·9 in negative samples except on one occasion. The amine test gave a positive result only when *C vaginale* was isolated. Comparison with 30 consecutive unselected patients confirmed the correlation between the clinical and laboratory diagnosis.

Tetracycline was effective in only half the patients treated, although 74% of the strains were sensitive in vitro, but metronidazole cured all but one of 17 patients eventually treated, although only 68% of the strains were sensitive. One of the nine placebo-treated patients was cured spontaneously.

Although the numbers examined were small, the clinical and laboratory correlation was stressed and the efficacy of metronidazole discussed in spite of the comparative in-vitro insensitivity.

R S Pattman

Sexual transmission of hepatitis A in homosexual men

L COREY AND KK HOLMES (University of Washington, Seattle, USA). *N Engl J Med* 1980; **302:** 435-8.

We performed monthly examinations and serological tests for antibody to hepatitis virus A (anti-HA) in a study of 57 heterosexual men and 102 homosexual men followed prospectively for a mean of 8·9 months and 6·1 months respectively. The initial prevalence of anti-HA was 30% in homosexual men and 12% in heterosexual men (P<0·01). The annual incidence of hepatitis A in susceptible (seronegative) homosexual men was 22%, whereas no heterosexual men acquired hepatitis A. Diaries concerning sexual behaviour kept by homosexual men showed that the acquisition of hepatitis A virus infection was correlated with frequent oro-anal sexual contact. Hepatitis A should be considered as one of the enteric infections that appear to be sexually transmitted among homosexual men.

Authors' summary

Extensive condylomata acuminata of the vulva treated by modified simple vulvectomy

GC TSOUTSOPLIDES (Berwick, Pennsylvania, USA). *Am J Obstet Gynecol* 1979; **135:** 1118-20.

Topical treatment of penile condylomata acuminata with colchicine at 48-72-hour intervals

G v-KROGH AND A-K RUDEN (Sodersjukhuset, Stockholm, Sweden). *Acta Derm Venereol* 1980; **60:** 87-9.

Intestinal parasites in homosexual men

A McMILLAN (Glasgow, UK). *Scot Med J* 1980; **25:** 33-5.

A study of the prevalence of intestinal parasites in a group of homosexual men, attending a sexually transmitted diseases clinic in Glasgow, was undertaken. Of 118 men examined over an eight-month period, four (one of whom had symptoms of dysentery) were found to be infected with *Entamoeba histolytica.* Cysts of *Iodamoeba buetschlii* were also found in the stool of one of these men. A further two patients had giardiasis and 11 men had enterobiasis. The importance of an awareness of these conditions is discussed.

Author's summary

Miscellaneous

Amoxycillin absorption and penetration in pelvic inflammatory disease

M ONSRUD, H GJONNAESS, AND T BERGAN (Aker Hospital, Oslo, Norway). *Acta Obstet Gynecol Scand* 1979; **58:** 401-3.

The efficacy of amoxycillin in five cases of laparoscopically diagnosed pelvic inflammatory disease was monitored. Pelvic penetration was determined by measurement of both peritoneal fluid and plasma concentrations of amoxycillin. Thin polyethylene catheters were introduced through the abdominal wall into the recto-vaginal pouch. (Microbiological isolation on culture was not reported.)

The mean peak concentration of amoxycillin in peritoneal fluid was 6μg/ml and similar to that in blood plasma. The mean lag in peak penetration of peritoneal fluid behind plasma was two hours. The lag was most marked in two severe cases of pelvic inflammatory disease with frank purulent peritoneal exudate. Therapeutic concentrations remained detectable for seven to eight hours after a single dose of 0·5 g of amoxycillin.

Monitoring was discontinued and the patients given a course of 0·5 g amoxycillin for 10-18 days three times daily. Although the five patients had patent Fallopian tubes at the time of the study, subsequent fertility had still to be tested.

J M Harvey

Risk of pelvic inflammatory disease among intrauterine-device users irrespective of previous pregnancy

S OSSER, B GULLBERG, P LIEDHOLM, AND N-O SJOBEG (University of Lund, Sweden). *Lancet* 1980; i: 386-8.

The use of intrauterine devices (IUD) in 690 patients admitted to hospital with pelvic inflammatory disease (PID or acute salpingitis) was compared with the use in a sexually active age-matched control group. Two hundred and twenty (31·9%) of the patients and 114 (16·5%) of the controls were using an IUD. Thus, the risk of PID was raised twofold by the use of an IUD. No significant difference was found between the women who had never been pregnant and those who had in the two groups. Neither was the risk of PID found to vary with age.

Authors' summary

Management of necrotizing vasculitis with colchicine—improvement in patients with Behcet's syndrome

PG HAZEN AND B MICHEL (University Hospital, Cleveland, USA). *Arch Dermatol* 1979; **115:** 1303-6.

Acute-phase proteins, C9, factor B, and lysozyme in recurrent oral ulceration in Behcet's syndrome

T LEHNER AND M ADINOLFI (Guy's Hospital, London, UK). *J Clin Pathol* 1980; **33:** 269-75.

Prostatism. I The correlation between symptoms, cystometric and urodynamic findings

JT ANDERSON, J NORDLING, AND S WATTER (University of Copenhagen, Denmark). *Scand J Urol Nephrol* 1979; **13:** 229-36.

Peri-urethral bacterial flora in women

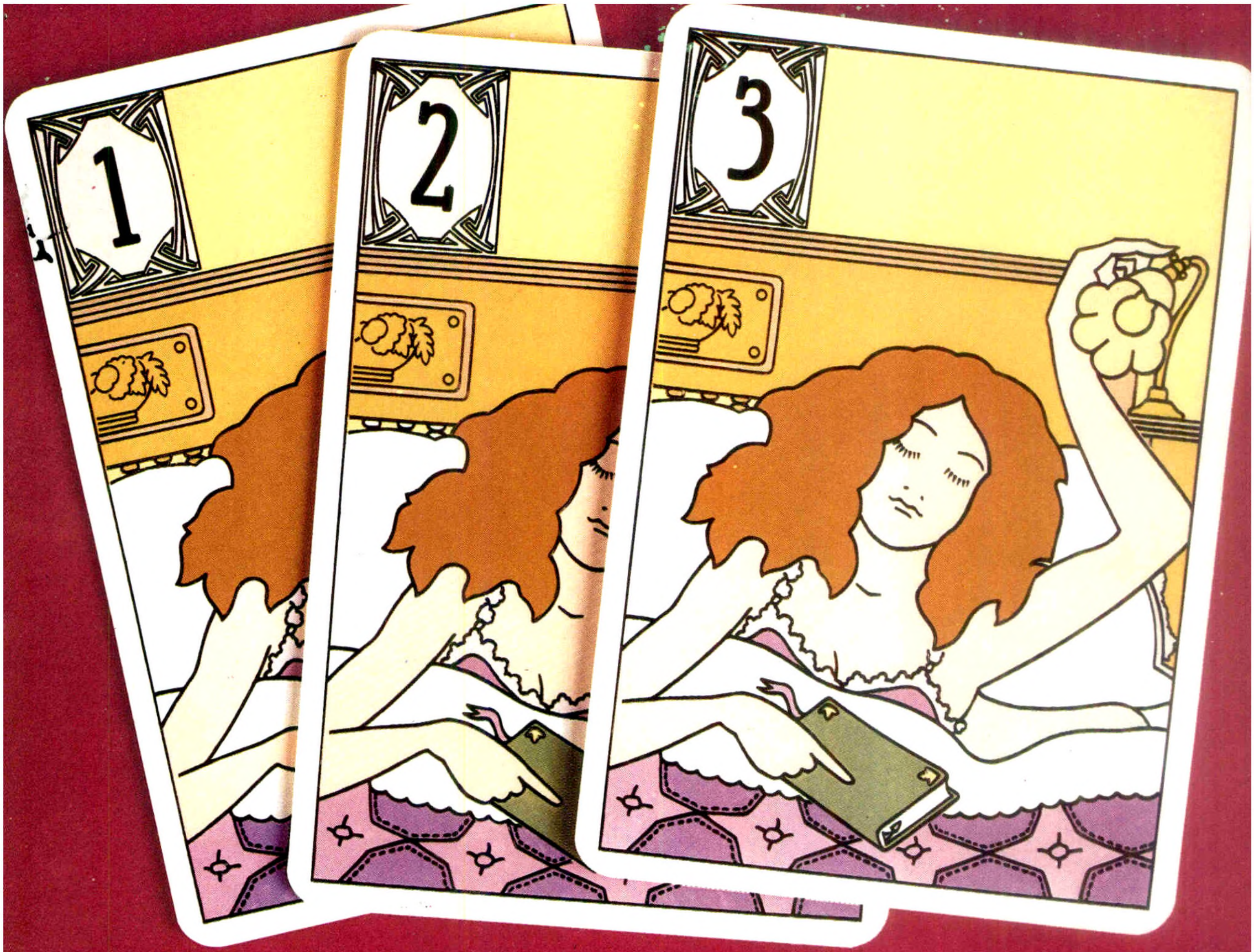
CM KUNIN, F POLYAK, AND E POSTEL (Ohio State University, USA). *JAMA* 1980; **243:** 134-9.

Vaginal parasitosis

MA GARUD, V SARALYA, M PARASKAR, AND J KHOKHAWALLA (Cama and Albless Hospital, Bombay, India). *Acta Cytol* 1980; **24:** 34-5.

Tampon-associated vaginal ulceration

EG FRIEDRICH AND KA SIEGESMUND (University of Florida, USA). *Obstet Gynaecol* 1980; **55:** 149-51.



FORTY-EIGHT HOUR PATIENCE

One tablet nightly for three nights means that your patient's patience only needs to last 48 hours. That's all it takes to clear vaginal candidiasis – twice as conveniently as the old One Two Free.

Canesten®
clotrimazole

New 200mg vaginal tablet now only one a night for three nights

Presentations Canesten is available either as 200 mg vaginal tablets in packs of three with applicator or as 100 mg vaginal tablets in packs of six with applicator. Canesten Duopak contains six 100 mg vaginal tablets with applicator plus 20 g cream containing 1.0% clotrimazole. **Dosage** *Vaginal Tablets 100mg* Two vaginal tablets should be inserted at night for three consecutive days. Alternatively, one vaginal tablet daily for six days. *Vaginal Tablets 200mg* One vaginal tablet should be inserted at night for three consecutive days. *Duopak* Vaginal Tablets 100 mg as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to prevent re-infection. **Side effects** Rarely patients may experience transient local mild burning or irritation. **Basic NHS Costs** 6 x 100 mg vaginal tablets with applicator £2.13; 3 x 200 mg vaginal tablets with applicator £1.99; Duopak £3.47. **Product Licence Numbers** Vaginal tablets 100 mg, PL 0010/0015; vaginal tablets 200 mg, PL 0010/0072; Duopak, PL 0010/0016 & PL 0010/0015. Further information is available from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. ® Registered

Sexually Transmitted Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $25.00 outside the United States ($22.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $28.00 in the United States and $30.00 in all other countries.

William O. Harrison, M.D.
American Venereal Disease Association
P.O. Box 22349
San Diego, California 92122 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $25.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases*.

☐ I wish to enter a Non-Member or institutional subscription ($28.00 U.S., all other countries $30.00).
(Mail non-member subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105)

Name ____________________

____

Address ____________________

City ____________________ State Country ____________ Zip ____________

British Journal of Venereal Diseases June 1980 Vol. 56 No. 3

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Influence of testicular fluid infected with *Treponema pallidum* on intradermal lesions
T J FITZGERALD AND R C JOHNSON *page* 125

Sulfhydryl oxidation using procedures and experimental conditions commonly used for *Treponema pallidum*
T J FITZGERALD, R C JOHNSON, AND E T WOLFF *page* 129

Ultrastructure of vascular and connective tissue changes in primary syphilis
TERESA WRZOŁKOWA AND JERZY KOZAKIEWICZ *page* 137

Identification of *Neisseria gonorrhoeae* in the routine venereological laboratory
T RUFLI *page* 144

Prevalence of cervical gonorrhoea in women with unwanted pregnancies
B VAN DER LUGT, A C DROGENDIJK, AND J R J BANFFER *page* 148

Comparative study of bacampicillin and ampicillin in the treatment of uncomplicated gonorrhoea
PATRICIA J SPENGLER AND LARRY D EDWARDS *page* 151

Quantitative aspects of chlamydial infection of the cervix
D HOBSON, P KARAYIANNIS, RUTH E BYNG, ELISABETH REES, I ANNE TAIT, AND JEAN A DAVIES *page* 156

Epidemiology of infection by serotypes D to K of *Chlamydia trachomatis*
ERIC M C DUNLOP, SOHRAB DAROUGAR, AND JOHN D TREHARNE *page* 163

Ureaplasma urealyticum in the urethra of healthy men
J VIARENGO, F HEBRANT, AND P PIOT *page* 169

Venereal diseases in the islands of the North Pacific
R R WILLCOX *page* 173

Sexually transmitted diseases: extract from the *Annual Report of the CMO of the DHSS for the Year 1978* *page* 178

Annual report of the Medical Society for the Study of Venereal Diseases *page* 182

Correspondence *page* 183

Book reviews *page* 185

Correction *page* 185

Abstracts *page* 186

ASTM CODEN: BJVDAK 56 (3) 125-191 (1980)

ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

Acc.
4.3.81

August 1980 Vol. 56 No. 4

British Journal of
Venereal Diseases

LIBRARY
C. U.

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

R N THIN *(Editor)*
J D ORIEL
B EVANS
A McMILLAN *(Abstracts)*
(Assistant Editors)
C B S SCHOFIELD
(President, MSSVD)

J BARROW
(Hon. Sec., MSSVD)
J WALLIN
G CAUSSE
J M KNOX
A E WILKINSON

I PHILLIPS
G CSONKA
E STOLZ
J THIVOLET
L M DRUSIN

EDITOR
British Medical Journal
SHUNA JUDGE
(Technical Editor)

This *Journal,* founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A E Wilkinson, 22 Kingsend, Ruislip, Middlesex HA4 7DA. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals,* available from the Publishing Manager, *British Medical Journal,* BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; **55**: 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS. *Venereal Diseases.* 3rd ed. London: Baillière and Tindall, 1975.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 214-22.

PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £23.00 in the United Kingdom and Republic of Ireland and US$55.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US$55.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal,* 1172 Commonwealth Avenue, Boston, Mass. 02134. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases,* BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1980 by the *British Journal of Venereal Diseases.* This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.

THEY CALL IT CRABS

You may know it as Phthirus pubis

Whatever you call them, pubic lice are a problem, because any measure you adopt to eradicate them must take care of both adult lice and nits, while being cosmetically acceptable. Quellada Shampoo fills the bill particularly well. Its active constituent, gamma benzene hexachloride, is rapidly lethal to crab lice, while the foaming shampoo base ensures that Quellada penetrates through to the nits. So a single application of Quellada kills lice *and* nits. Quellada Shampoo is easy and pleasant to use, because it doesn't smell or irritate the skin.

Quellada PC Shampoo

gamma benzene hexachloride 1%

kills pubic lice and nits in 4 minutes.

Presentation
Gamma benzene hexachloride 1% in a specially formulated foaming shampoo base.

Uses
Antiparasitic and ovacidal. For use on hairy areas of the body in the treatment of lice infestations, e.g. head lice, body lice, pubic lice.

Dosage and administration
Apply to wet hair, rub vigorously for four minutes, rinse and comb with fine-tooth comb.

Contra-indications, Warnings etc.
Avoid contact with the eyes. Should lather accidentally get into eyes, flush immediately with water. Do not use more than twice in any one infestation nor in any one week. Do not use on broken or infected skin.

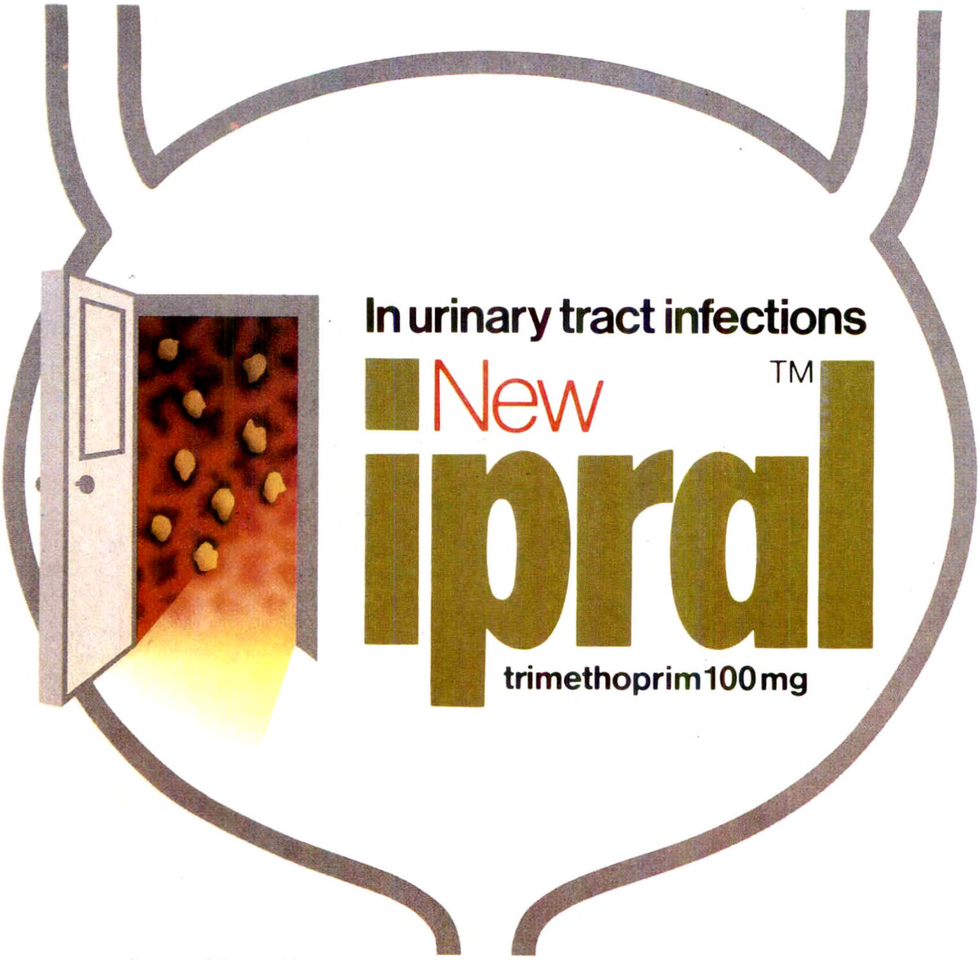
Packaging and price
Bottles of 100 and 500ml. Basic NHS cost 60p for 100ml.

Product Licence No.
0036/5001.

Further information is available on request.

Stafford-Miller Limited,
Professional Relations Division,
Hatfield, Herts. AL10 0NZ.

UNIVERSITY LIBRARY



In urinary tract infections

New ipral™

trimethoprim 100 mg

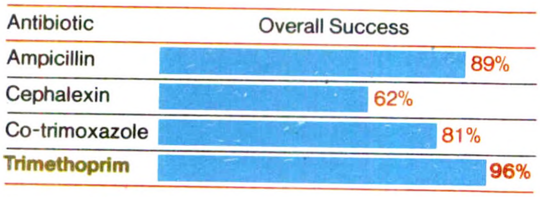
...the way to effective treatment of urinary tract infections

Trimethoprim – proved effective as a single entity without the side-effects associated with sulphonamides.

"Trimethoprim alone may well prove as effective as trimethoprim-sulphamethoxazole combinations ..."[1]

96% success in general practice.[2] Half as many side-effects.

Results of 107 Courses of Treatment in 96 Domiciliary Patients



Antibiotic	Overall Success
Ampicillin	89%
Cephalexin	62%
Co-trimoxazole	81%
Trimethoprim	**96%**

ipral simple treatment for acute infections – 2 tablets b.d.

ipral for routine use in chronic U.T.I. – 1 tablet b.d.

In a study on recurrent U.T.I.'s
"... trimethoprim is the most effective of the antibacterial agents examined."[3]

Greater efficacy with fewer side effects.

In the trial in acute U.T.I.'s

"... the total side effects caused by ampicillin, cephalexin and co-trimoxazole were similar and occurred twice as often as when trimethoprim was given alone ..."[2]

Antibiotic	No. of Patients	% Side Effects
Ampicillin	88	22
Cephalexin	84	25
Co-trimoxazole	83	21
Trimethoprim	**84**	**8**
Total	339	

Prescribing information

IPRAL tablets
Each tablet contains trimethoprim B.P. 100 mg

Indications
Ipral is indicated for the treatment and prophylaxis of acute and chronic urinary tract infections.

Actions
Ipral is active in-vitro against most pathogenic Gram-positive and Gram-negative bacteria. Exceptions include Neisseria species and Pseudomonas aeruginosa.

Dosage and administration
For acute urinary tract infections 2 tablets twice daily is recommended. For long term and prophylactic therapy 1 tablet twice daily is recommended.

Contra-indications
Ipral should not be given to patients with severe renal insufficiency where blood levels cannot be monitored regularly. Ipral should not be used in pregnancy or during the first few weeks of life.

Precautions
Care is necessary in administration to patients with impaired renal function. Special precaution should be exercised in patients with a predisposition to folate deficiency.

Side-effects
Nausea, vomiting and skin rashes may occur but are rare. Ipral may depress haematopoiesis during prolonged therapy. This has proved reversible when treated with calcium folinate.

Treatment of overdosage
Gastric lavage. Calcium folinate may be used to counteract any effect of Ipral on bone-marrow.

Package quantities and price
Packs of 100 tablets £5.30.
Product Licence No. – 0034/0190.

Full prescribing information available from:
Technical Services Department, E. R. Squibb & Sons Ltd.
Regal House, Twickenham, TW1 3QT
Tel: 01-892 0164

5280 Oct 79

Ipral is a Trade Mark of E. R. Squibb & Sons Ltd.

...the way to effective treatment of urinary tract infections

- As effective as trimethoprim/sulphamethoxazole combination
- Avoids the side effects of sulphonamides
- At a sensible price

and "Trimethoprim given alone was shown to be much better tolerated."[2]

"Treatment with TMP (trimethoprim) alone is superior to the combined regimen due to a smaller incidence of side-effects; ..." thus "... introduction of TMP alone on the market is recommended."[4]

"Less gastrointestinal side-effects and skin rashes have occurred with trimethoprim ..."[3]

References
1. Lancet 1979, **1**, 493. 2. Br. med. J. 1972, **2**, 673.
3. Ann. clin. Res. 1978, **10**, (suppl. 22). 4. Chemother. 1973, **19**, 314.

New
ipral™
trimethoprim 100 mg
more than half the story

™
SQUIBB Full prescribing information available from Technical Services Department, E. R. Squibb & Sons Ltd. Regal House, Twickenham, TW1 3QT Tel 01-892 0164

CLINICAL PRACTICE IN SEXUALLY TRANSMISSIBLE DISEASES

Robertson, McMillan and Young

This book is written primarily for those engaged in the clinical practice of venereology but will also be of use to general practitioners and other clinicians involved in counselling and giving contraceptive advice. The necessity of a sympathetic approach to the patients and to the tracing of contacts is emphasised for this type of infection which involves deep personal feelings of all concerned.

Clinical problems, such as prostatitis and frequency/dysuria in young adults, have been discussed fully and in each chapter the problems in therapy and the toxicity of currently used drugs are described. Space is also given to sexually transmissible diseases to give an appreciation of Herpesvirus, Hepatitis B virus, Cytomegalovirus and other infections.

D. H. H. Robertson MB, ChB, FRCPEd, DTM&H. Head of University of Edinburgh Department of Venereal Diseases and Consultant Venereologist, Edinburgh Royal Infirmary.

A. McMillan MB, ChB, BSc, MRCP(UK). Honorary Senior Lecturer, University of Edinburgh Department of Venereal Diseases, Edinburgh Royal Infirmary. Consultant Venereologist, Greater Glasgow Health Board.

H. Young BSc, PhD. Lecturer in Bacteriology, University of Edinburgh, Department of Bacteriology with an Honorary appointment to Department of Bacteriology, Royal Infirmary of Edinburgh.

229 x 152 mm 468 pages Illustrated 0 272 79568 2 £27.00 net Cased

ORDER NOW from your local bookseller or in case of difficulty from the Publisher

Pitman Medical

57 High Street, Tunbridge Wells, Kent TN1 1XH

M.S.S.V.D.

The Medical Society for the Study of Venereal Diseases

11 Chandos Street, Cavendish Square, London W1M 0EB
President: Dr. E. M. C. Dunlop

The object of the Society is to bring together those interested or engaged in the diagnosis, treatment, and public health aspects of venereal diseases. The Society meets regularly in London for the reading of papers, discussions, and presentation of cases. An out-of-London meeting is held in the Spring.

Annual subscription for membership of the Society is £12 or $22.00 for all members.

Applications for membership are very welcome and forms can be obtained from the Honorary Secretary, Dr. J. Barrow, Lydia Department, St. Thomas' Hospital, SE1 7EH.

Programme 1980-81

Friday October 31st	Short papers	
Friday November 28th	The T.P.I. test	Dr. A. E. Wilkinson Dr. J. B. Selkon Professor J. Thivolet Dr. J. L. Fluker
Friday 30th January, 1981	Intestinal Parasites	Professor Lewis M. Drusin Dr. A. Macmillan Dr. J. W. Acers
Friday 27th March, 1981	Vulval Disease	Professor Charles Douglas
Friday 24th April, 1981	Presidential Address Higher Medical Training in Venereology	

For the comprehensive management of non-gonococcal urethritis

Deteclo

Tetracycline hydrochloride, Chlortetracycline hydrochloride,
Demeclocyline hydrochloride

for first line therapy

**Deteclo will resolve the symptoms of NGU in the majority of cases.
One tablet b.d. for 21 days gave 88.6% success in NGU[1]**

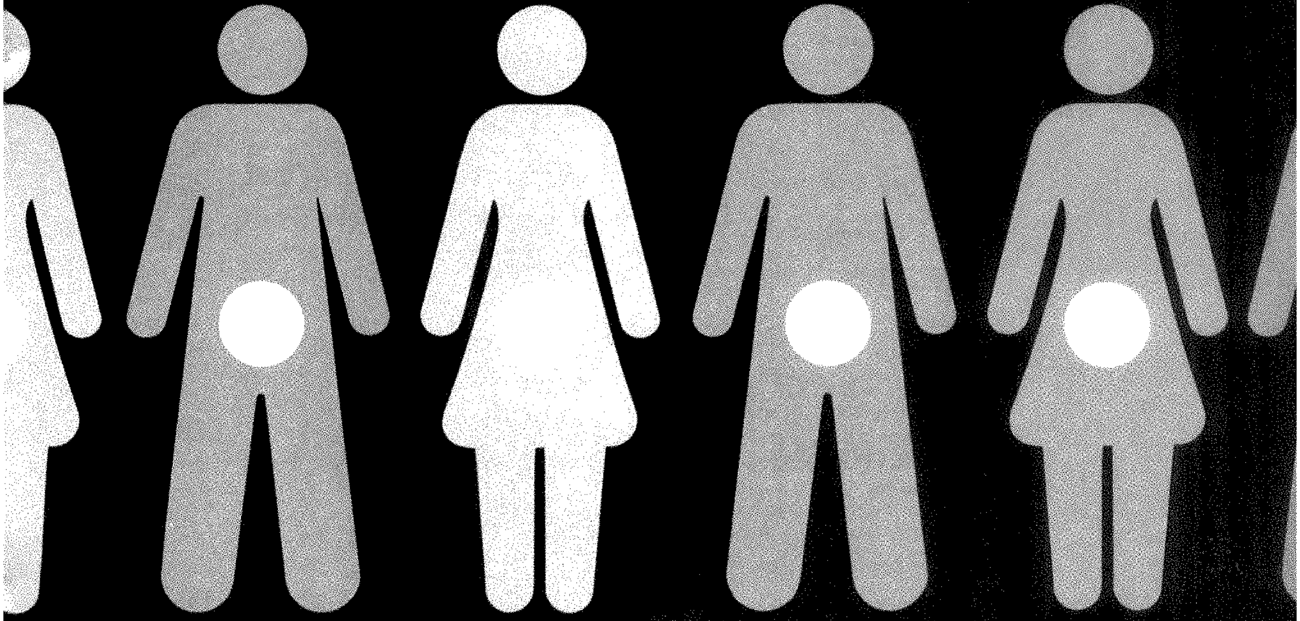
. . . but for the 1 patient in 4 who presents with recurrent NGU, Minocin is the logical choice for rapid resolution of symptoms.

The seven day treatment course, exceptional activity against chlamydia[2] and ureaplasma[3] and the simple b.d. dosage make MINOCIN the treatment of choice in cases of recurrence.

Minocin

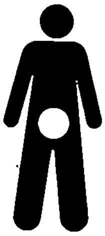
Minocycline hydrochloride

for recurrent NGU



Lederle

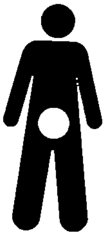
For Prescribing Information see over page



Deteclo

for first line therapy

Prescribing information
Uses
Venereal Diseases. Non-Gonococcal Urethritis. Urinary tract infections. Other infections due to DETECLO-sensitive organisms.
Dosage
Adults only.
Non-Gonococcal Urethritis: One tablet twice daily for 10-21 days.
Gonorrhoea: 1200 mg (4 tablets) followed by a similar dose six hours later
Other Infections: One tablet every 12 hours. This may be increased to three or four tablets daily for short periods in more severe infections.
Contra-Indications
Known tetracycline hypersensitivity. Overt renal insufficiency. Systemic lupus erythematosus.
Precautions
Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts. Lower doses are indicated in cases of renal impairment to avoid excessive systemic accumulation, and, if therapy is prolonged, serum level determinations are advisable.
Warnings and side effects
Gastro-intestinal disturbances are the side effects most commonly reported. A yellow to brown discolouration of teeth is a possible sequela of treatment during pregnancy. Photoallergic reactions may occur in hypersensitive persons.
Legal category
POM
Presentation
Each blue 300 mg tablet printed 'Lederle 5422' contains: tetracycline hydrochloride 115.4 mg, chlortetracycline hydrochloride 115.4 mg, demeclocycline hydrochloride 69 2 mg. Supplied in bottles of 100 and 500 tablets at a basic NHS cost of £7.59 per 100 tablets.
(correct at time of going to press)
Product licence number Tablets PL/0095/5070
DETECLO is a Trademark



Minocin

for recurrent NGU

Prescribing information
Uses
Venereal Diseases. Non-Gonococcal Urethritis Urinary tract infections. Prostatitis. Salpingitis Other infections due to MINOCIN-sensitive organisms.
Dosage
Adults only.
Non-Gonococcal Urethritis: One tablet twice daily for a minimum of 7 days.
Gonorrhoea: In adult males, 300 mg as a single dose. Adult females may require more prolonged therapy.
Other Infections: Two tablets initially followed by one tablet twice daily.
Unlike earlier tetracyclines, absorption of MINOCIN is not significantly impaired by food or moderate amounts of milk.
Contra-Indications
Known tetracycline hypersensitivity. Systemic lupus erythematosus.
Precautions
Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts.
Warnings and side effects
Gastro-intestinal disturbances are the side effects most commonly reported. Dizziness may occur. A yellow to brown discolouration of teeth is a possible sequela of treatment during pregnancy.
Legal category
POM
Presentation
Orange tablets printed 'Lederle 5334' containing 100 mg of minocycline as the hydrochloride. Supplied in packs of 9 and 45 tablets at a basic NHS cost of £15.78 per 45 tablets.
(correct at time of going to press)
Product licence number Tablets 100mg PL/0095/0006
MINOCIN is a Registered Trademark
Full prescribing information is available on request

Lederle Laboratories
A division of Cyanamid of Great Britain Ltd
Fareham Road, Gosport, Hants PO13 0AS

1. Thambar, I V, et al, Brit J Vener Dis 1979; 55 (4), 284–288
2. Bowie, W R, et al, J Infect Dis 1978, 138 (5) 655–659
3 Spaepen, M S, et al, Antimicrob Ag Chemother 1976, 9 (6): 1012–1018

Br J Vener Dis 1980;**56**:193.

Editorial

Gonorrhoea: a continuing problem in the 1980s

Recently, attention was drawn to the problem of β-lactamase-producing gonococci in the United Kingdom.[1] These organisms were first reported in the UK in 1976,[2,3] and most reports have been of single sporadic cases. However, there was an outbreak in Liverpool in 1976 in which β-lactamase gonococci were isolated from 76 patients during the period from February to November of that year.[4] These organisms had plasmids with a molecular weight similar to that found in isolates originating in Ghana and this may have been the origin of the outbreak in Liverpool, although epidemiological studies suggested that the outbreak may have originated in the city itself.[4] Apart from this outbreak, most of the isolates found in the United Kingdom have been imported from West Africa or the Far East.[1] Laboratories have been requested to report such isolates, but only nine were reported in 1977 and 29 in 1978 whereas 25 were reported in the first six months of 1979.[1]

So far, reports from other countries have concerned sporadic cases. However, reports in this issue[5-7] show that these strains are becoming an increasing problem numerically in the Netherlands, accounting for 2-3% of all circulating gonococcal strains.[5] Furthermore, these β-lactamase strains appear to have established themselves in the local population—mainly in prostitutes—and imported strains accounted for only 14% of those identified in the first half of 1979.

In addition, β-lactamase-producing strains comprise 40% of isolates in some parts of the USA[8] and Singapore.[9] They are present in prostitutes in Hong Kong[10] but appear to be less common in Lübeck, West Germany.[11]

The management of gonorrhoea continues to present a challenge. Initial antimicrobial treatment must be based on what is known of the probable sensitivity of the infecting strain of gonococcus. Careful follow up and rapid and vigorous contact tracing, especially where antimicrobial resistance is suspected, remain essential. It is vital to monitor antimicrobial sensitivity, to send all isolates with suspected β-lactamase production to a reference laboratory, and to disseminate information concerning resistance.

In the 1970s, there was a marked increase in the prevalence of gonorrhoea in most parts of the world, although there has been some levelling off in the UK during the second half of the decade.[1] During the 1970s β-lactamase-producing strains were isolated and in 1979 they appear to have increased in number. What will the 1980s bring?

R N THIN

References

1. Sexually transmitted disease surveillance 1978. *Br Med J* 1979;ii:1375-6.
2. Phillips I. β-lactamase-producing, penicillin-resistant gonococcus. *Lancet* 1976;ii:656-7.
3. Percival A, Rowlands J, Corkill JE, *et al.* Penicillinase-producing gonococci in Liverpool. *Lancet* 1976;ii:1379-82.
4. Arya OP, Rees E, Percival A, Alergant CD, Annels EH, Turner GC. Epidemiology and treatment of gonorrhoea caused by penicillinase-producing strains in Liverpool. *Br J Vener Dis* 1978;**54**:28-35.
5. Bijkerk H. Penicillinase-producing gonococci in the Netherlands. *Br J Vener Dis* 1980;**56**:243.
6. Nayyar KC, Noble RC, Michel MF, Stolz E. Gonorrhoea in Rotterdam caused by penicillinase-producing gonococci. *Br J Vener Dis* 1980;**56**:244-8.
7. Nayyar KC, Michel MF, Stolz E. Antibiotic sensitivities of gonococci isolated in Rotterdam and results of treatment with cefuroxime. *Br J Vener Dis* 1980;**56**:249-51.
8. Berg SW, Kilpatrick ME, Harrison WO, McCutchan JA. Cefoxitin as a single-dose treatment for urethritis caused by penicillinase-producing *Neissena gonorrhoeae*. *N Engl J Med* 1979;**301**:509-11.
9. Rajan VS, Sng EH, Pang R, Tan NJ, Thirumoorthy T, Yeo KL. HR 756: A new cephalosporin, in the treatment of gonorrhoea caused by ordinary and penicillinase-producing strains of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1980;**56**:255-8.
10. Wang FH. β-lactamase-producing gonococci in Hong Kong. *Br J Vener Dis* 1980;**56**:270.
11. Grunder K, Petzoldt D. Sensitivity of *Neisseria gonorrhoeae* to spectinomycin and thiamphenicol. *Br J Vener Dis* 1980;**56**:252-4.

Br J Vener Dis 1980;**56**:194-7.

Sexually transmitted diseases in Nigeria

R D CATTERALL
From James Pringle House, the Middlesex Hospital, London

Background

Nigeria is a young, rapidly developing country. Its boundaries were drawn by the British in 1900 but it has only been independent since 1960. Its surface area is about four times that of Great Britain and it has an estimated population of between 80 and 100 millions. Geographically it can be divided into four zones. The coastal belt includes extensive sandy beaches, behind which are mangrove swamps. Thick tropical rain forests extend inland for over 100 miles to give way to a grassy savannah alternating with tree-covered parkland. The north is continuous with the Sudan savannah zone and merges into the Sahara desert. The main activity of the people throughout Nigeria is farming.

The climate ranges from equatorial in the south to tropical in the middle zones and arid in the north. Temperatures are high. On the coast there is little variation from 28 to 30°C with a relative humidity of over 70% whereas in the north, in the dry season, temperatures may reach 43°C during the day and fall to 4°C at night. The humidity will average about 30%.

Rainfall is seasonal. The dry season varies from three months in the south to six months in the north. The rainfall decreases from south to north so that Lagos, on the coast, receives over 2000 mm a year whereas Sokoto in the north has about 690 mm.

Nigeria is the most highly populated state in Africa and the ninth most populous in the world.

THE PEOPLE

There are more than 250 ethnic groups, as the area was one of the focal points of the many large population movements in Africa. The largest groups are the Hausa, principally in the north, the Yoruba, in the south-west, and the Ibo, in the south east, who together make up 70% of the total. The population has been increasing very rapidly but accurate figures are difficult to obtain. The south of the country is much more densely populated than the north. Urbanisation has been occurring at a phenomenal rate and the number and size of cities has increased rapidly, associated with recent economic development and increasing prosperity.

For administrative purposes the country is divided into 19 states. Lagos is the federal capital, and Nigeria's main port, as well as being the centre for industrial, commercial, and financial affairs. Its population is well over three million and it is said to be one of the fastest growing cities in Africa. Recently the government has announced that the capital is to be transferred from Lagos to a new site in the centre of the country.

The second largest city in Nigeria is Ibadan, with a population of well over two million inhabitants. It is an important industrial centre situated in the middle of a cocoa-producing area and has a well known university. Kano is the largest town in the north and is an ancient trading centre with rapidly growing modern industry, including a motor-car assembly plant.

English is the official, commercial, and main cultural language. It is the medium of education at all levels. About 45% of the population are Muslim, predominantly in the north, over 30% are Christian (divided between Catholics, Protestants, Methodists and Presbyterians), mainly in the south, and some of the remainder follow a variety of traditional beliefs.

THE GOVERNMENT

On 1 October 1963, Nigeria became a federal republic within the Commonwealth. In 1966 there was a military coup and several leading politicians were massacred. From 1967 to 1970 there followed a terrible civil war with uncontrolled killings and starvation. After the war a military government remained in office and the present regime has arranged to return power to a civilian government in October 1979 after new elections.

LEGAL SYSTEM

The legal system is based on English law. There is a federal supreme court, presided over by the Chief Justice, which is the final court of appeal. It has power to interpret the Constitution and hears disputes over legal rights between state governments,

Address for reprints: Dr R D Catterall, James Pringle House, the Middlesex Hospital, London W1N 8AA

Received for publication 16 October 1979

or between a state and the federal government. Each state has a high court. As in Britain, the magistrates courts have jurisdiction in a wide variety of civil and criminal cases.

ECONOMY

Agriculture was the basis of the Nigerian economy until recently and still provides a livelihood for nearly 70% of the population. The main crops are cocoa, groundnuts, cotton, rubber, and palm produce, and the main food crops are rice, maize, guinea corn, millet, and yams. Nigeria is the world's largest producer of cocoa and the largest producer of rubber in Africa. Oil was first discovered in 1956 and the rate of production increased rapidly in the ensuing 24 years. Nigeria is now the world's seventh largest oil producer and the largest in Africa. Most of it is found in the delta of the River Niger and in the offshore fields. Natural gas, coal, tin, and iron ore are other riches found in the country, which could secure its economic progress well into the next century.

EDUCATION

Education has a long tradition of Christian missions in the south whereas in the north there is a strong Islamic tradition. Government policy is aimed at providing free, universal, and compulsory primary education in all areas. Nearly all secondary schools are fee-paying. In 1960 there were two universities but by 1979 there were 12. The oldest is the University of Ibadan, which was originally constituted in 1948 as a college affiliated to the University of London.

The illiteracy rate however is still high in Nigeria and is believed to affect about 75% of the population, in spite of literacy programmes. The government is trying to involve young people in the development of the country as a whole and has launched a National Youth Service Corps which qualified students must join. They are sent to all parts of the country to teach, do medical work, or organise voluntary labour.

HEALTH CARE

Most tropical diseases are endemic in Nigeria. The prevalent diseases are malaria, tuberculosis, dysentery, meningitis, measles, and tetanus. Other major health problems are hepatitis A, intestinal parasites, trypanosomiasis, onchocerciasis, and sexually transmitted diseases. Leprosy is still common but yaws now appears to be rare.

Public health facilities, including hospitals and clinics, are run by the states although there are a few federal institutions. Government health facilities are free to government employees and are inexpensive to everyone else. The current plan is to try to achieve a more equitable distribution of health services, which only reach about 50% of the population. It is planned to establish 285 basic health units throughout the country.

Patients have to pay for medical consultations, investigations, and treatment. There are some general practitioners, mostly in the urban areas, but many patients just turn up at a hospital. They are interviewed by a fee collector who assesses how much the patient can afford to pay and are then sent to a sorting room to be assigned to a clinic.

Patients who are to be admitted to hospital have to deposit 100 Naira (about £60) and usually have to pay about 20 Naira a day. Paupers and those unable to pay are treated free of charge.

The hospital service is financed by the state departments of health and the university hospitals by the Ministry of Education. Many of the university hospitals are very new or just developing. There is a shortage of general hospitals and of state-sponsored specialist hospitals. In many of these there may be over 500 new outpatients every day and one doctor may see as many as 90 patients a day.

Outside the main cities there are district hospitals with 30 to 40 beds and one doctor. An increasing number of health centres and dispensaries with nurses and attendants are being developed.

The British left behind them a number of mission hospitals, which are highly regarded, and there are an increasing number of private clinics and hospitals run by private doctors and commercial firms. In more outlying areas there are some mobile dispensaries. The St Johns Ambulance Brigade is active in Nigeria and there is a Nigerian Red Cross Society.

Taking the country as a whole, most patients are treated with traditional remedies and medicines, but an increasing number treat themselves with antibiotics and other modern drugs, which are freely available to everyone over the counter of chemists shops, in markets, and from merchants. Most have had some type of self-medication before they consult a doctor or go to a hospital.

Sexually transmitted diseases

There are only four clinics for patients with sexually transmitted diseases in the whole of Nigeria for a population of nearly 80 million people. They are in Ibadan, Lagos, Enugu, and Zaria.

The prevalence of sexually transmissible infections is unknown but is believed to be high, especially in the rapidly developing new towns. Self-treated patients probably form the largest group but in the rural areas traditional remedies are known to be popular. Inadequate treatment is the rule, but provided the symptoms are controlled most patients seem satisfied. This is probably an important factor in the development of β-lactamase-producing strains of gonococci in West Africa.

In the University College Hospital special clinic in Ibadan, non-specific urethritis is the commonest disease to be diagnosed, followed by gonorrhoea, trichomoniasis, and syphilis. Vaginal candidosis is diagnosed less frequently than in Britain, but the other sexually transmitted diseases are seen with a similar distribution to other countries.

Immediate Gram-stained smears are available and cultures are performed on selective media. The Venereal Disease Research Laboratory test and *Treponema pallidum* haemagglutination assay are used for serological investigation and *Trichomonas vaginalis* is looked for in a wet preparation. Darkground microscopy is also readily available.

Treatment schedules are similar to those used in Britain but benzathine penicillin is used exclusively in the treatment of syphilis in all stages. Chancroid is usually treated with sulphonamides and lymphogranuloma venereum with tetracyclines.

Contact tracing is enthusiastically carried out despite enormous difficulties. Casual contacts, reluctance to reveal names, unnamed streets, unnumbered houses, and the rapid movement of the population provide many problems. Nevertheless, the clinic is run efficiently and has an atmosphere resembling a busy active centre in Britain.

Patients with sexually transmitted diseases are frequently seen in general and specialist medical and surgical clinics as well as in private practice. They are usually treated with antibiotics without an accurate diagnosis; no contact tracing is attempted and no follow-up visits are arranged.

To test the market, personal experience is often best. In a large market in the centre of Ibadan I approached a merchant who was surrounded by remedies for all types of human ailments. I told him I had a discharge and that I thought it might be gonorrhoea and asked him what he recommended. Without hesitation he put his hand in his pocket and produced four black and red capsules which he offered to me. The price was 2 Naira each capsule (about £1) and I was instructed to take all the capsules at once. When I suggested that I might need more than four capsules, I was told that four were quite enough and I was reassured in a confident and convincing manner. When I said that I should like to seek another opinion, I was told it was quite unnecessary and my reception became decidedly less friendly.

The Future

Nigeria is a vast heavily populated country which is developing and changing at a phenomenal rate. The wealth resulting from the rapid increase in production of oil has been used for many prestige developments such as public buildings, universities, large well equipped armed forces, and rapid industrial expansion. There are however serious flaws in the present structure of the country because so many of the public services do not work. Powercuts are frequent, telephones do not function, telegrams take weeks to be delivered, the roads are full of pot-holes and are not maintained, there are serious delays in administrative action, and bribery is widespread in all walks of life.

Much of this is not surprising when the speed of change since independence only 19 years ago is taken into account. The medical services have expanded enormously but it must be remembered that this is an area of Africa with serious indigenous diseases, such as malaria, intestinal parasites, and trypanosomiasis. There is not yet a safe drinking-water supply in urban or country areas and sewage disposal is primitive and often non-existent. A great deal of basic preventive medicine remains to be done and has become urgent as a result of the rapid growth of the towns throughout the country.

As far as the sexually transmitted diseases are concerned, there is no doubt that they are very common, and many new factors—such as greater freedom for women, the increasing use of non-occlusive contraceptives, greater mobility with more frequent travel, and the absence of a nationwide network of clinics—must all result in a further increase in their prevalence and in greater problems resulting from the complications of inadequately treated diseases and the absence of contact tracing.

Nevertheless, a start has been made and the model clinic at University College Hospital, Ibadan, created by Professor A O Osoba, has now been copied at three other university centres. The pattern that is emerging is that the clinic is part of the department of medical microbiology and the clinics are staffed by members of that department, who are also responsible for the microbiological and serological support service. Trainees are normally sent to Britain for postgraduate training, and to obtain diplomas in microbiology and venereology, after adequate experience at home. This type of organisation may well be the best solution to the problem until it is economically possible to establish a separate service for patients with sexually transmitted diseases run by physicians with specialist training and devoting all their time to the specialty.

The rest of the world will watch the development of Nigeria and other West African countries with sympathy, understanding, and some anxiety, because of the regular export of β-lactamase-producing strains of gonococci to the outside world. No country wants to be a bad neighbour, but if it is exporting antibiotic-resistant strains of micro-organisms to its friends and neighbours, it may quickly earn that reputation. The problem is urgent and appears to be increasing in magnitude. There are signs however that since the very successful First African International Conference on Sexually Transmitted Diseases held in Ibadan in August 1979 governments and health authorities in Africa are more aware of the situation and may be prepared to allocate greater resources to try to contain these infections.

Br J Vener Dis 1980;56:198-203.

Pleural effusion disease agent as passenger of Treponema pallidum suspensions from rabbits

Survey of laboratories

KNUD L FENNESTAD, LIS BRUUN, AND ERIK WEDØ
From the Animal Department, Statens Seruminstitut, Copenhagen, Denmark

SUMMARY Material from rabbits used for the propagation of *Treponema pallidum* in 12 selected laboratories was examined for a viral passenger agent of the treponemal suspensions. An agent which causes clinical or subclinical pleural effusion disease (PED) in rabbits and which is serologically identical or related to the PED agent isolated in Copenhagen was found as a contaminant in treponemal suspensions in laboratories in Europe, the USA, and Japan. The experience gained in the Scandinavian treponematoses laboratories suggests that strains of *T pallidum* contaminated with the PED agent can be purified by passage through hamsters.

Introduction

Pleural effusion disease (PED) is an intercurrent infection among rabbits used for the propagation of pathogenic treponemes. The infection was originally recognised as an intercurrent mortality problem in Scandinavian laboratories using Nichols pathogenic strain for the *T pallidum* immobilisation (TPI) test.[1-3]

The same disease appears to have emerged independently under similar circumstances in France,[4] and there is some evidence to suggest that a subclinical form of the disease occurs in the USA.[5] At present, however, PED appears to be confined to treponematoses laboratories.

The aetiological agent of PED is considered to be a virus which is transmitted as a passenger of the treponemal suspension, but as yet it has not been demonstrated by culture, electron microscopy, or by a specific serological technique.[6] Experiments in rabbits show that the agent can be demonstrated regularly in the circulating blood for 2-30 days after infection. The pathogenicity for rabbits can be varied by manipulation from fatal to subclinical disease. After infection the PED agent multiplies rapidly in the host, reaching titres of about 10^5 rabbit-infective doses per ml of blood within a couple of days. After 3-4 weeks protective antibodies become demonstrable.[8]

PED is considered to be a "new" rabbit disease, characterised clinically by fever, lymphocytopenia, leucocytosis, anaemia, iridocyclitis, anorexia, and death. The most conspicuous finding at necropsy is large amounts (up to 50 ml) of plasma-like fluid in the pleural cavities, hence the name pleural effusion disease.[8] The histopathological findings are minimal. Fatal infections are characterised by reduction of the splenic white pulp and focal degeneration of the thymus and lymph nodes. Later in the infection there is hyperplasia of the splenic white pulp and an increased amount of interstitial follicular lymphoid tissue in the lungs.[9 10]

Various methods have been proposed to alleviate the problem of intercurrent rabbit mortality.[4 11 12] Passage of the rabbit testicular suspension of treponemes through hamsters and back to rabbits appears to remove the PED agent from the treponemes,[11] and this principle has been applied as a practical solution in some of the Scandinavian laboratories.

The widespread use of treponemes propagated in rabbits for various serological tests and for experimental purposes prompted the present investigation for the PED agent in rabbit material from selected treponematoses laboratories. The investigation also provided an opportunity to study the Scandinavian experience with hamster passages of contaminated Nichols strains.

Materials and methods

PARTICIPATING LABORATORIES

Fifteen laboratories from 11 countries were asked to supply material. The laboratories were selected on

Address for reprints: Dr K L Fennestad, Statens Seruminstitut, Amager Boulevard 80, 2300 Copenhagen S, Denmark

Received for publication 4 October 1979

TABLE I *Survey of treponematoses laboratories from which paired rabbit sera were received**

Laboratory		*T pallidum strain used*		*History of rabbit mortality*	*Hamster passages of treponemal strain*	*Paired sera*	
No	*Location*	*Designation*	*Received from*			*Date*	*No of rabbits*
Category (a)							
1	Copenhagen	Nichols	London (1972)	+	+	4/4/1979	4
2	Stockholm	Nichols	Copenhagen (1954)	+	+	20/12/1978	6
3	Olso	Nichols	Copenhagen (1969)	+	0	17/10/1978	4
4	Helsinki	Nichols	Copenhagen (1973)	+	+	27/3/1979	4
5	Paris	Nichols	Baltimore (1950)	+	0	8/12/1978	5
6	Utrecht	Nichols	Copenhagen (1975)	+	0	1/11/1978	4
	Utrecht	Utrecht	Local strain (isolated 1967)	+	0	1/11/1978	4
Category (b)							
7	Wrocław	Nichols	Copenhagen (1958)	0	0	9/5/1979	4
8	Tokyo	Nichols	Copenhagen (1967)	0	0	21/7/1979	5
Category (c)							
9	Los Angeles	Nichols	Baltimore (1950)	0	0	26/3/1979	2
10	London	Nichols	London (1952†)	0	0	15/12/1978	4
Category (d)							
11	Atlanta	Nichols	Chapel Hill NC (1961)	0	0	6/2/1979	4
12	Minneapolis	Nichols	Atlanta (1973)	0	0	19/3 and 31/7/1979	4

*Information from laboratories were given in personal communications as follows: No 1, Jørgensen BB (1970-79); No 2, Hederstedt B (1974-79); No 3, Erichsen S, Loe KG (1974-79); No 4, Aho K (1971-79); No 5, Vaisman A, Paris A (1978); No 6, Kruijt BC, Menke HE (1978); No 7, Metzger M (1979); No 8, Sugahara T, Nakagawa M (1979); No 9, Miller JN (1979); No 10, Wilkinson AE (1978); No 11, Clark JW (1979); and No 12, Johnson RC (1979).

†From C W Chacko, St Mary's Hospital Medical School.

+Positive, 0 negative

the basis of (a) a history of present or past excessive rabbit mortality; (b) having received a Nichols strain from the Copenhagen laboratory without subsequent increased rabbit mortality; (c) having supplied the Copenhagen laboratory with a Nichols strain; and (d) having no mortality problem or exchange of strains with the Copenhagen laboratory.

One laboratory in each of categories (a) and (d) did not respond within the time limit, and one in category (c) was unwilling to supply any material for examination. Table I gives a survey of the participants, which included three of the four WHO collaborating centres dealing with research in treponematoses.

SERUM SAMPLES

Each laboratory was asked to send frozen paired sera from 2-4 rabbits obtained before and 48 hours after intratesticular infection with treponemes. Freeze dried sera were received from two laboratories (Nos 8 and 12), and from another (No 11) two of the four postinfection sera were not obtained until 96 hours after infection. One preinoculation specimen was lost during shipment (No 2). On arrival all sera were stored at −70°C in aliquots of 0·5 ml.

Information was obtained on the origin of the *T pallidum* strain currently used, the total dose of treponemes given to the rabbits, and whether or not the strain in question had been maintained in animal species other than the rabbit. The Scandinavian laboratories, including the Finnish laboratory, supplied more detailed information.

The choice of paired rabbit sera had several objectives. Examination of the normal rabbit serum before inoculation with treponemes served to demonstrate the presence or absence of the PED agent. Examination of the 48-hour postinoculation serum was used to demonstrate the presence or absence of the PED agent in the treponemal suspension given to the rabbit. The primary reason for examining the 48-hour postinfection rabbit serum rather than the treponemal suspension—which is routinely prepared in the second week after treponemal infection—was the enrichment effect secured by giving the PED agent a chance to be in the logarithmic phase of growth.[6]

DEMONSTRATION OF THE PED AGENT

Animals

Conventional albino rabbits originating from the closed colony at Statens Seruminstitut (Ssc:CPH) are considered to be free from PED infection.[8] Observations to the contrary have never been made since the discovery of PED in the early 1960s. Male rabbits weighing 1900-2900 g were used for all inoculations. Before use, these animals had been employed once for pyrogen testing of protein fractions of human blood.

Method of examination

A 0·2-ml quantity of each serum specimen mixed with 0·8 ml PBS (pH 7·0) was inoculated subcutaneously into one rabbit. The rabbit was then observed for fever, iridocyclitis, loss of weight, and death for a period of 10 days. Twenty days after the end of the observation period, all surviving rabbits were challenged by subcutaneous inoculation of 10^3 rabbit-infective doses of the virulent Copenhagen PED agent[6] and observed for another 10 days in the same manner as before. Animals that died were examined as described previously.[8] In five cases the test was repeated either because the rabbit died intercurrently (two animals) or because the necropsy findings did not exclude convincingly a cause of death other than the PED infection (three animals).

Fever together with iridocyclitis or death with characteristic necropsy findings of PED or both after the inoculation were considered as evidence of the presence of the PED agent in the specimens. Challenge 30 days after the serum inoculation served to demonstrate the presence or absence of immunity to the Copenhagen PED agent.

Results

EXAMINATION OF RABBIT SERA BEFORE TREPONEMAL INFECTION

The results of rabbit inoculations with 53 serum specimens from 12 laboratories and the results of the subsequent challenge of these animals with the PED agent are shown in table II.

Only one out of the 53 specimens gave a positive reaction in the rabbit test (table II). This specimen is considered to have been contaminated inadvertently with a postinfection serum from the same laboratory (No 3). Titration of content of rabbit-infective doses in the suspect pair of sera and determination of interferon concentration in all eight specimens (kindly performed by Dr Sv Haahr, University of Aarhus) support this contention.

When the inoculated animals were challenged with the Copenhagen PED agent, all except the abovementioned rabbit proved to be susceptible to infection and developed clinical signs or showed necropsy findings typical of PED or both. This indicates that none of the rabbits used by the laboratories was harbouring the PED agent before infection with treponemes.

EXAMINATION OF RABBIT SERA AFTER TREPONEMAL INFECTION

The 54 postinoculation sera originated from rabbits

TABLE II *Results of rabbit inoculation with "normal" serum followed by challenge with PED agent 30 days later*

Preinoculation sera			
Laboratory	*No of rabbits inoculated*	*With clinical evidence of PED*	*With clinical protection after challenge*
Category (a)			
1	4	0/4	0/4 (3 died)
2	5	0/5	0/5 (5 died)
3	4	1/4	1/4 (2 died)
4	4	0/4	0/4 (2 died)
5	5	0/5	0/5 (3 died)
6*	4	0/4	0/4 (3 died)
	4	0/4	0/4 (2 died)
Category (b)			
7	4	0/4	0/4 (3 died)
8	5	0/5	0/5 (3 died)
Category (c)			
9	2	0/2	0/2 (2 died)
10†	4	0/4	0/4 (4 died)
Category (d)			
11	4	0/4	0/4 (1 died)
12	4	0/4	0/4 (1 died)

*Represented by two strains of *T pallidum*.
†Two of the four specimens from rabbits given cortisone.

which had each received an intratesticular total dose of $1{\cdot}6\times10^6$ to 2×10^8 treponemes. In seven laboratories the inocula were treponemal suspensions prepared on two to four different dates. In each of the remaining five laboratories the rabbits were probably inoculated with the same suspension.

Table III shows that a positive clinical reaction for PED infection occurred in rabbits inoculated with material from six laboratories (Nos 2, 3, 5, 6, 7, and 8). With the exception of laboratory No 2, surviving animals from this group challenged with the PED agent all proved to be protected, thus indicating that immunity to the Copenhagen PED agent had developed.

Rabbits inoculated with serum specimens from laboratory No 12 showed no clinical signs of disease except for transient fever in two of the four animals. Nevertheless, all these four rabbits were completely protected when challenged, thus indicating the presence in the specimens of an agent of low virulence conferring immunity to PED infection.

A problematic response to inoculation and challenge was observed with the specimens from laboratory No 2. Clinical signs of PED were observed in three of the six animals, but in these three animals the onset of iridocyclitis was atypically delayed. On challenge, one of the six animals developed clinical signs of PED and two of the other five animals became febrile.

No clinical evidence of PED infection could be demonstrated in any of the specimens from five laboratories (Nos 1, 4, 9, 10, and 11), and on challenge all rabbits were found to be susceptible to the Copenhagen PED agent.

REMOVAL OF THE PED AGENT FROM CONTAMINATED NICHOLS STRAINS BY PASSAGES IN HAMSTERS

In the 1960s, the four Scandinavian TPI laboratories experienced high mortality among rabbits inoculated with treponemes. The Copenhagen and Stockholm laboratories carried out passages of their Nichols strains in hamsters to remove the agent causing death among the rabbits while the Oslo laboratory made no such change. The Helsinki laboratory received hamster-passaged Nichols strains from Copenhagen.

The date of initiation of hamster passages, the number of passages, the date of the resumption of the passages in rabbits, and the results of the present examination for the PED agent are shown in table IV.

None of the initial passages of the Nichols strains in hamsters was successful in any of the three laboratories. Admittedly, the rabbit mortality was reduced immediately, but intercurrent deaths typical of PED occurred within one to two years. After the latest passage in hamsters, it would appear that the Helsinki and Copenhagen laboratories have been free from PED contamination for more than five and two years respectively. In the same periods the annual mortality in these two laboratories has been close to zero.

The Stockholm laboratory apparently harbours an

TABLE III *Results of rabbit inoculation with postinoculation serum followed by challenge with PED agent 30 days later*

Postinoculation sera			
Laboratory	*No of rabbits inoculated*	*With clinical evidence of PED*	*With clinical protection after challenge*
Category (a)			
1	4	0/4	0/4 (1 died)
2	6	3/6	5/6
3	4	3/4 (2 died)	2/2
4	4	0/4	0/4 (2 died)
5	5	4/5 (3 died)	2/2
6*	4	3/4 (2 died)	2/2
	4	4/4 (1 died)	3/3
Category (b)			
7	4	3/4	4/4
8	5	4/5	5/5
Category (c)			
9	2	0/2	0/2 (2 died)
10†	4	0/4	0/4 (3 died)
Category (d)			
11	4	0/4	0/4
12	4	0/4	4/4

*Represented by two strains of *T pallidum*.
†Two of the four specimens from rabbits given cortisone.

agent in spite of the hamster passages, but the mortality has been less than 1% during the last three years.

The PED agent still exists in the Oslo laboratory, and the annual mortality during the last three years has varied from 4 to 12%.

It would appear from the results that passages of the Nichols strains through hamsters and back to rabbits were not convincingly successful in removing the contaminating agents in Stockholm and Copenhagen. As yet there are no explanations for these failures, but from the experience in Copenhagen it seems that recontamination may also occur in the laboratory when contaminated and non-contaminated treponemal suspensions for rabbit inoculation are prepared in the same room.

Discussion

Four types of reactions could be differentiated in the rabbit test used. A negative reaction, observed with the preinoculation specimens from all laboratories and also with the postinoculation specimens from five laboratories, shows that neither clinical nor immunological evidence of the PED agent could be demonstrated. The question as to whether previously acquired antibodies against the PED agent were present in these negative specimens cannot be shown by the rabbit test, since undoubtedly such antibodies in the small inoculum used no longer exerted a protective effect at the time of challenge.

A strong positive reaction was observed with the postinoculation specimens from five laboratories (Nos 3, 5, 6, 7, and 8). Inoculation of these specimens resulted in clinical signs or death typical of PED infection or both. The result of challenge shows that the causative agents were serologically identical or closely related to the PED agent isolated in Copenhagen. It is reasonable to conclude that the agents demonstrated after inoculation with treponemes were also present in the suspension of treponemes given to the rabbits, since no evidence of PED infection could be demonstrated in the normal sera obtained before the treponemal inoculation.

Apparently the more pathogenic PED strains were isolated from laboratories which had had high rabbit mortality (Nos 3, 5, and 6), and the less pathogenic strains from laboratories with no history of mortality (Nos 7 and 8). Such a difference in virulence is in accordance with experimental observations on the PED infection.[8]

From this point of view, the third type of reaction is not surprising. The only evidence of PED infection demonstrated in material from one laboratory (No 12) was the development of immunity, thus suggesting a subclinical infection with the PED agent. This strain with a low virulence is now being passed serially in rabbits to be observed for increase of virulence.

The fourth type of reaction was encountered with the postinfection specimens from the Stockholm laboratory (No 2). The provisional interpretation of this reaction is that the agent demonstrated is related to but not identical with the PED agent.

It would appear that the repeated passages in hamsters did not remove this agent from the Nichols strain in Stockholm (table IV). Gudjónsson and Skog,[3] reporting in 1970 on the contamination of the Swedish Nichols strain, mention that the findings in the intercurrent disease in Stockholm were similar to

TABLE IV *Results of hamster passages of contaminated Nichols strains in Scandinavian TPI laboratories*

Laboratory	*Hamster passages*		*Transfer back to passages in rabbits*	*Examination for PED*	
	Onset	*No*		*Date*	*Result*
Copenhagen	Apr 1970	5	Sep 1970		
	Sep 1970	1	Oct 1970		
	Nov 1972	2	Jan 1973*		
	Aug 1976	2	Oct 1976	Apr 1979	0
Helsinki	Apr 1970	5†	Sep 1970‡		
	Nov 1972	2†	Jan 1973§	Mar 1979	0
Stockholm	Aug 1970	1	Nov 1970		
	May 1971	3	Oct 1971		
	Mar 1975	2	Aug 1975		
	Dec 1975	2	May 1976	Dec 1978	+?
Olso				Oct 1978	+

*Rabbit passages temporarily interrupted by storage of treponemes in liquid nitrogen.
†Hamster passages carried out in Copenhagen.
‡Helsinki received twentieth rabbit passage from Copenhagen in April 1971.
§Helsinki received eighth rabbit passage from Copenhagen in October 1973.
+ Positive, 0 negative

those observed in Copenhagen.[1] Therefore, our observations raise the question as to whether the so-called "Stockholm agent" is identical with the agent examined in the present rabbit test.

It should be remembered that the Stockholm agent was also studied at the International Treponematoses Laboratory in Baltimore. A challenge experiment made there caused Gudjónsson *et al*[5 12] to suggest that the local Nichols strain in Baltimore might be contaminated by an agent similar to the Stockholm agent.

The present isolation of an agent from the Minneapolis laboratory conferring immunity to challenge with the PED agent makes this suggestion more credible.

Furthermore, in this survey, the isolate from Minneapolis may represent the most recently acquired contamination of treponemes, provided that the Atlanta Nichols strain was also free from the PED agent in 1973 when the strain was received in Minneapolis.

It has been suggested that the PED agent may be present subclinically for 10 or 20 years in rabbit passages of the Nichols strain.[8] This assumption is supported by the demonstration of the PED agent in material from two laboratories (Nos 7 and 8) which received the Nichols strain from Copenhagen three years before and six years after the recognition of rising intercurrent rabbit mortality in Copenhagen in 1961.

The importance of the PED agent as a cause of rabbit mortality has been reported previously.[1 8] This survey shows that the PED agent may also exist as a silent or unrecognised contaminant in other laboratories which maintain the Nichols strain in rabbits.

The significance of the subclinical contamination of rabbit-passaged strains of *T pallidum* is not known. It should be pointed out that a concomitant PED infection may possibly interfere not only with yield of treponemes from rabbit testes but also with other host reactions, which may lead to hazards in interpretation of experimental results.

Addendum

A recent article by Small *et al*[13] describes the manifestations of rabbits infected with the Stockholm agent, brought to Baltimore in 1970 from Stockholm.[5] The clinical and histopathological observations by these authors are essentially the same as previously described for the PED agent.[8-10] This is in agreement with the notion that the agent present in Stockholm in 1978 is no. longer identical with the Stockholm agent.

References

1. Jorgensen BB. Spontaneous deaths among rabbits inoculated with *Treponema pallidum* less than 2 weeks before. *Z Versuchstierk* 1968; **10**:46-54.
2. Erichsen S. Discussion of B B Jorgensen's paper. *Z Versuchstierk* 1968; **10**:46-54.
3. Gudjónsson H, Skog E. Fever after inoculation of rabbits with *Treponema pallidum*. *Br J Vener Dis* 1970; **46**:318-22.
4. Vaisman A, Paris-Hamelin A, Catalan F, Dunoyer F. Entretien au laboratoire des souches de tréponèmes pathogènes. Precautions à prendre. *La Prophylaxie Sanitaire et Morale* 1973; **45**:71-3.
5. Gudjónsson H, Newman B, Turner TB. Demonstration of a virus-like agent contaminating material containing the Stockholm substrain of the Nichols pathogenic *Treponema pallidum*. *Br J Vener Dis* 1970; **46**:435-40.
6. Fennestad KL, Haahr S, Bruun L. Pleural effusion disease in rabbits. Interferon in body fluids and tissues after experimental infection. *Acta Path Microbiol Scand (B)* 1979; **87**:311-5.
7. Fennestad KL, Jensen H-JS, Møller S, Bentzon MW. Pleural effusion disease in rabbits. Clinical and post-mortem observations. *Acta Path Microbiol Scand (B)* 1975; **83**:541-8.
8. Christensen N, Fennestad KL, Bruun L. Pleural effusion disease in rabbits. Histopathological observations. *Acta Path Microbiol Scand (A)* 1978; **86**:251-6.
9. Fledelius H, Bruun L, Fennestad KL, Andersen SR. Uveitis in rabbits with pleural effusion disease. Clinical and histopathological observations. *Acta Ophthalmol (Kbh)* 1978; **56**:599-606.
10. Jensen H-JS. Elimination of intercurrent death among rabbits inoculated with *Treponema pallidum*. *Acta Path Microbiol Scand (B)* 1971; **79**:124-5.
11. Gudjónsson H, Newman B, Turner TB. Screening out a virus-like agent from the testicular suspension of the Nichols pathogenic *T pallidum*. With observations on certain characteristics of the agent. *Br J Vener Dis* 1972: **48**:102-7.
12. Small JD, Aurelian L, Squire RA, Strandberg JD, Melby EC Jr, Turner TB, Newman B. Rabbit cardiomyopathy. Associated with a virus antigenically related to human Coronavirus strain 229E. *Am J Path* 1979; **95**:709-29.

Br J Vener Dis 1980;**56**:204-9.

Venereal diseases in the islands of the South Pacific*

R R WILLCOX
From the Department of Venereology, St Mary's Hospital, London

SUMMARY The island territories of the South Pacific vary considerably in area and in size of population; Pitcairn has a population of 100 in two square miles whereas Papua New Guinea has a population of 2 990 000 in approximately 175 000 square miles. Today the whole ocean is traversed by air routes. Recently, the prevalence of gonorrhoea has decreased in the northern region but increased in the eastern and western; in all these regions the reported prevalence exceeds 200 cases per 100 000 population. In an area where yaws was once widespread, syphilis is being increasingly recognised. Although the figures for syphilis are clearly higher because of the greater use of serological screening, many of the reported cases are of early infection. Yaws has been eliminated from most of the South Pacific Islands but is still present in the western region—more than 99% of the reported cases occurring in Papua New Guinea, particularly in the offshore islands.

Introduction

The reported cases of venereal diseases in the Pacific islands north of the equator have already been considered.[1] International health statistics for the islands of the South Pacific are published by the World Health Organisation and by the South Pacific Commission, a consultative and advisory body set up in 1947 by the six governments then responsible for the administration of the various island territories—namely, Australia, France, the Netherlands, New Zealand, the United Kingdom, and the USA. Since that time the Netherlands have ceased to participate, but as an increasing number of countries achieve independence new participating members are being added—Western Samoa (1964), Nauru (1969), Fiji (1971), Papua New Guinea (1975), and the Gilbert Islands (Kiribati) (1979).

POLITICAL ATTACHMENTS

Of the 20 islands, or groups of islands, providing data to the South Pacific Commission (figure), five lie in the northern region (of which Guam, the Trust Territory of the Pacific Islands, and half of the Gilbert Islands are north of the equator), six in the eastern region, and nine in the western region, corresponding broadly to Micronesia, Polynesia, and Melanesia respectively. Their political attachments are shown in table I.

COMMUNICATIONS

Tourism is vital to the economy in many Pacific islands and is fostered by the airlines which serve them (table II). International air routes in the South Pacific run in a north-east to south-west direction from the USA—by way of Honolulu, or Nadi (Fiji), or both, but less often by way of Rarotonga in the Cook Islands or Pago Pago in American Samoa—to New Zealand and Australia. A transverse route links Papeete (Tahiti) in French Polynesia, Nadi (Fiji), and Noumea in New Caledonia (also French), with extensions on both sides and northwards also through Nauru. These key centres are linked by international flights connecting the islands with the Americas and with Australasia, but these and other airports also provide local flights between adjacent island groups and different points on the island groups themselves. For example, in Papua New Guinea alone (table II), 72 places are served by scheduled air routes (DZ, GV, JX and PX); in French Polynesia (from Papeete, Tahiti), 22 (VT and QE); in the Solomon Islands (from Honiara on Guadalcanal), 22 (IE); in Fiji (from Suva), 12 (FJ and PC); in New Caledonia (from Noumea), 12 (TY); in the New Hebrides (from Espiritu Santo and Port Vila), 11 (IE); in Tonga (from Tongatapu), four (HK); in Western Samoa (from Apia), four (PH); in Guam and the Mariana Islands, three (IL); and in the Cook Islands (from Rarotonga), two (KH).

---

*Paper presented at the meeting of the Medical Society for the Study of Venereal Diseases, Lübeck, West Germany, May 1979

Address for reprints: Dr R R Willcox, Tideway, Lonsdale Road, London SW13

Received for publication 4 October 1979

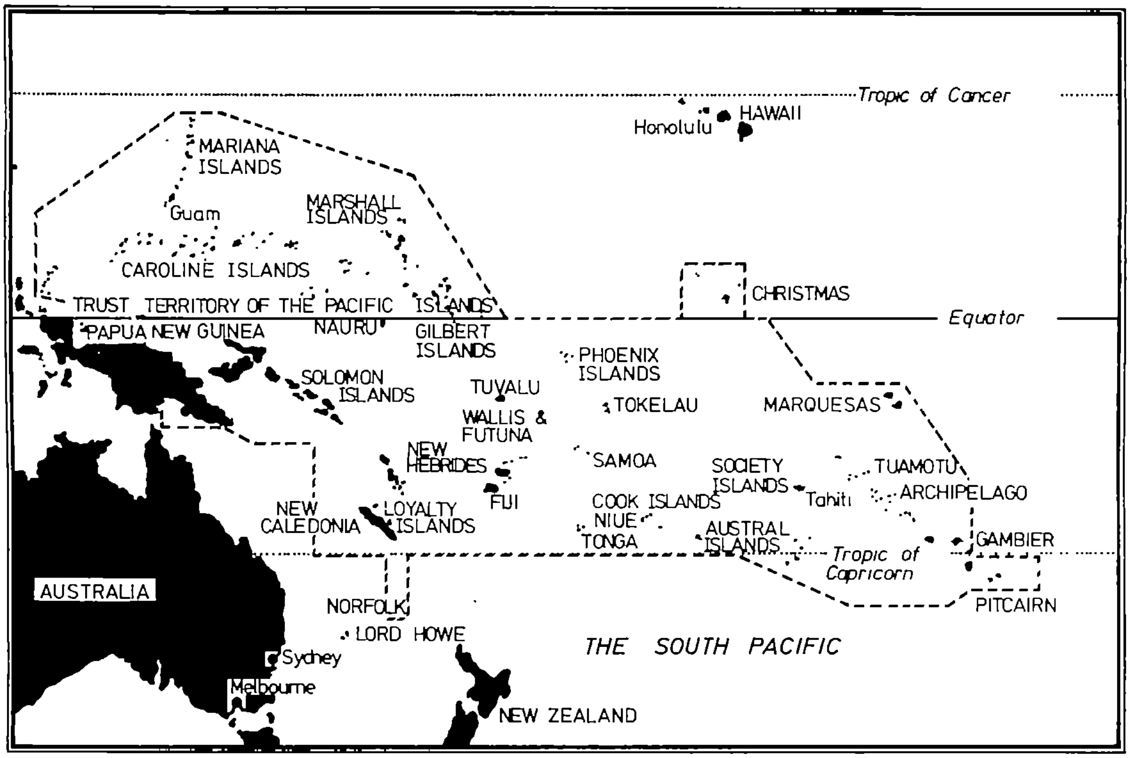


FIGURE *Islands of the South Pacific*

TABLE I *Political attachments of Pacific Islands south of the equator*

Political attachment	*Pacific Islands**
Independent	Fiji (UK) Kiribati (Gilbert Islands, UK) Nauru (Australia) Niue (New Zealand) Papua New Guinea (Australia) Solomon Islands (UK) Tonga (UK) Tuvalu (Ellice Islands, UK) Western Samoa (New Zealand)
Australia	Lord Howe Islands Norfolk Island
Great Britain	New Hebrides (with France) Pitcairn Islands (including Ducie and Veno atolls and Henderson Island)
Chile	Easter Island San Felix Juan Fernandez
Ecuador	Galapagos Islands
France	French Polynesia New Caledonia New Hebrides (with Britain) Wallis and Futuna Islands
New Zealand	Cook Islands Tokelau

*Previous adminstration given in brackets

TABLE II *Airlines serving the Pacific Islands south of the equator*

*Airlines**
External:
Air New Zealand (TE)
Canadian Pacific Air (CP)
Cathay Pacific Airlines (CX)
Lan Chile (LA)
Pan American World Airways (PA)
Qantas Airways (QF)
Union de Transports Aeriens (UT)
Internal:
Advance Airlines (DR)
Air Caledonie (TY)
Air Melanesiae (HB)
Air Nauru (ON)
Air Niugini (PK)
Air Pacific (FJ)
Air Polynesie (DZ)
Air Tahiti (QE)
Bougair (JX)
Cook Island Airways (KH)
Douglas Airways (DZ)
East-West Airlines (EW)
Fiji Air (PC)
Norfolk Island Airways (UG)
Polynesian Airlines (PH)
Solomon Island Airways (IE)
South Pacific Island Airways (HK)
Talair (GV)

*Codes as per *Official Airline Guide*[15]

DEMOGRAPHY

The three regions (northern, eastern, and western) have a total population of 4·8 millions, of which 62·3% (or nearly three millions) live in the single large territory of Papua New Guinea, which covers approximately 175 000 (85·4%) of the 205 000 square miles of the consolidated land mass.

Northern region

In the northern region the Trust Territory of the Pacific Islands has both the largest land area and the greatest population (133 000) distributed among those of its 1350 or so coral islets which are inhabited and which extend 4000 miles from east to west and lie just north of the equator; the equally scattered Gilbert Islands have the second largest land area. Both Nauru and Tuvalu cover 10 square miles or less with populations between 7000 and 7400 (table III).

Ocean Island (in the Gilbert Islands) and Nauru, both of which lie just south of the equator, have been heavily mined for phosphates. The rich inhabitants of the latter have a very high prevalence of diabetes.

Eastern region

The eastern region covers more than 2·5 times the land area of the northern region and has approximately 1·5 times its population (table III).

The most scattered group of islands, covering some 2·75 million square miles of ocean, is that of French Polynesia, comprising the Marquesa Islands, the Society Islands, the Tuamotu Archipelago, and the Austral and Gambier Island complexes. Their total population is exceeded by that of Western Samoa, a more compact group of two large and seven small islands. The smallest islands in this region are the three coral atolls of Tokelau (population 1600) and Pitcairn Island (and its adjoining atolls) with a population of only 100.

The Galapagos Islands (not a member of the South Pacific Commission) lie almost on the equator to the eastern edge of the region on the same latitude as the coast of Ecuador, to which they belong.

Western region

The western region is the largest region in terms of population and land area (both population and size being disproportionately exaggerated by the presence of Papua New Guinea). Even excluding Papua New Guinea, the population and size of the remaining territories are several times greater than those of the other two regions (table III).

The western region contains rich mineral deposits of copper in Bougainville, lately separated from Papua New Guinea, and of nickel in New Caledonia.

TABLE III *Land area and population size of South Pacific Islands*

Region	*Estimated population (1978)*	*Land area*	
		km^2	*Square miles*
Northern			
TTPI	133 000	1832	707
Guam	90 000	541	209
Gilbert Islands	56 000	690	266
Tuvalu	7400	26	10
Nauru	7000	21	8
Total	293 400	3110	1200
Eastern			
French Polynesia	141 000	3265	1260
Western Samoa	153 000	2935	1133
Tonga	93 000	699	270
American Samoa	31 500	197	76
Cook Islands	18 500	240	93
Wallis and Futuna	10 000	255	98
Niue	3700	259	100
Tokelau	1600	10	4
Pitcairn	100	5	2
Total	452 400	7865	3036
Western			
Papua New Guinea	2 990 000	452 943	174 880
Fiji	607 000	18 272	7055
Solomons	214 000	28 530	11 015
New Caledonia	138 000	19 103	7374
New Hebrides	101 500	11 880	4586
Norfolk Island	1900	34	13
Total	4 052 400	530 762	204 923
Total (excluding Papua New Guinea)	1 062 400	77 819	30 043

Venereal diseases statistics

Statistics for syphilis and gonorrhoea in the Pacific Islands are collected and published by the World Health Organisation (together with those in Australia and New Zealand, under the heading of Oceania); the rates are provided by the South Pacific Commission.

The prevalence of both diseases are subject to the usual variables, such as the availability of diagnostic facilities and the accuracy of reporting. Quite often there is a difference (sometimes considerable) in the figures reported by the two international organisations, the reasons for which are not clear.

PREVALENCE OF GONORRHOEA

The prevalence of gonorrhoea in the entire Southern Pacific area (191 per 100 000 in 1975) was exceeded—among reportable communicable diseases—only by measles and influenza (table IV). In many parts of the region however the diagnosis of gonorrhoea may only be a clinical one and is seldom based on the results of cultures.

Northern region

In a previous paper,[1] data from the WHO indicated a rising prevalence of gonorrhoea in Guam and the

TABLE IV *Communicable disease in the Pacific Islands*[6]

Disease	*Rate**	*Disease*	*Rate**
Influenza	3006	Tuberculosis	24
Measles	419	Other meningitis	16
Gonorrhoea	191	Yaws	12
Dengue	170	Meningococcal meningitis	9
Bacillary dysentery	100	Rheumatic fever	8
Amoebic dysentery	73	Tetanus	5
Fish poisoning	39	Leprosy	3
Pertussis	38	Encephalitis	2
Infective hepatitis	35	Typhoid fever	2
Syphilis	34	Poliomyelitis	1
		Diphtheria	1

*Per 100 000 population

Trust Territory of the Pacific Islands with peaks in 1972 and 1973[3] (G Causse, personal communication, 1978). Data from the South Pacific Commission[5-8] indicate that in the five-year period from 1973 to 1978 the reported number of cases of gonorrhoea decreased but the overall prevalence is still substantial at 266 per 100 000 (having decreased from 438 per 100 000), being particularly high in Guam and in the widespread Trust Territory of the Pacific Islands, where the reported prevalence in 1978 was 374 per 100 000 (table V).

TABLE V *Prevalence of gonorrhoea in the South Pacific Islands*

	No of cases				
Region	*1973*	*1974*	*1977*	*1978*	*Rate* (1978)*
Northern					
TTPI	756	592	479	498	374
Guam	411	270	218	258	287
Nauru	6	4	9	9	129
Gilberts	25	0	30	13	23
Tuvalu	†	†	0	1	14
Total No of cases	1198	866	736	779	
Rate per 100 000	438	303	259	266	
Eastern[5-8]					
Cook Islands	202	156	174	120	649
French Polynesia	226	264	367	408	289
American Samoa	49	48	79	69	219
Western Samoa	240	267	184	290	190
Tokelau				1	188
Niue	7			1	81
Wallis and Futuna	1	1		4	40
Tonga	22	18	3	19	20
Total No of cases	747	754	807	912	
Rates per 100 000	175	172	182	202	
Western[5-8]					
Papua New Guinea	5156	5759	8954	10 386	347
Fiji	811	490	930	1073	177
New Caledonia	165	228	331	244	177
Norfolk Island			29	3	158
Solomon Islands	12	9	377	194	91
New Hebrides	122	99	96	66	65
Total No of cases	6266	6585	10 717	11 966	
Rate per 100 000	202	184	272	295	

*Per 100 000 population
†Included in figures for Gilbert Islands

Eastern region

In the eastern region of the South Pacific Commission the prevalence of gonorrhoea in 1978 also exceeded 200 per 100 000, the highest rate of 649 per 100 000 being returned (as before when it was even higher) by the Cook Islands (table V). Unlike in the northern region, however, the rate had increased from 175 per 100 000 (or by 15·6%) since 1973 (table V).

Western region

The World Health Organisation's figures[3 4] for gonorrhoea show an increasing prevalence in Fiji between 1955 and 1971 (with a peak of 1506 cases) and a subsequent decrease to 930 cases in 1977; similarly, the number of cases in New Caledonia increased from 127 in 1968 to 398 in 1976 whereas in the New Hebrides the number rose from 59 cases in 1970 to 276 in 1976.

In data from the South Pacific Commission[5-8] the highest regional rate of 295 per 100 000 was reported from the western region, which showed an increase of 63·9% over five years (table V).

Papua New Guinea, with the highest rate in 1978 of 347 per 100 000, accounted for 86·8% of the reported cases of gonorrhoea in this region (82·3% in 1973).

It is noteworthy that the three cases among the small population of 1900 in Norfolk Island in 1978 gave a prevalence of 158 per 100 000. In the previous year, when there were 29 cases, the prevalence was 1526 per 100 000.

PREVALENCE OF SYPHILIS

Regional figures

Venereal syphilis is emerging as a public health problem in the South Pacific, where yaws was once endemic.[9]

The prevalence in the three regions is shown in table VI. Few cases are notified in the returns each year from the northern region. (No case of syphilis was reported in the Trust Territory of the Pacific Islands up to 1972.) In the eastern region, a high prevalence has been reported from French Polynesia (319 per 100 000 in 1978) whereas the three cases reported in Tonga in 1978 were the first ever recorded in that territory.

In the western region the highest rate in 1978 was 299 per 100 000 in New Caledonia but in 1977 there were 462 cases per 100 000.[7] This rate, like that of French Polynesia, showed almost a 10-fold increase in the rate since 1973. The number of cases in Papua New Guinea (which accounted for 74·9% of the grand total of 4652 cases) had also increased more than threefold since 1973, during which time a 100-fold increase was noted in Fiji.

UNIVERSITY LIBRARY

TABLE VI *Syphilis in the South Pacific*[5-8]

Region	*No of cases* 1973	1974	1977	1978	*Rate** *(1978)*
Northern					
Guam	3	0	4	2	2
TTPI	4	1	5	0	0
Other islands†	0	0	0	0	0
Eastern					
French Polynesia	31	76	316	450	319
Tonga	0	0	0	3	3
Other islands‡	0	0	0	0	0
Western					
New Caledonia	31	219	628	412	299
Papua New Guinea	1021	1153	1937	3485	117
Fiji	3	30	143	300	49
New Hebrides	1	0	2	0	0
Solomon Islands	0	0	1	0	0
Norfolk Island	0	1	0	0	0
Total No of cases	1094	1480	3036	4652	
Rates per 100 000	26	34	65	97	

*Per 100 000 population
†Gilbert Islands
‡American Samoa, Cook Islands, Niue, Pitcairn Island, Tokelau, Wallis and Futuna, and Western Samoa

Type of case

Although the number of cases of syphilis has increased more than fourfold, many are latent, discovered as a result of the increasing facilities for serological testing—as in French Polynesia, New Caledonia,[7] and Fiji.

Moreover, 78 cases of syphilis were reported in New Caledonia in 1955 but the annual number fell to five in 1970 before rising again (table VII). Similarly, in Fiji, 48 cases were recorded in 1955—but only three or four in 1966 and 1973—the number then increased rapidly to 143 in 1977.[4] Nevertheless, numerous cases of clinical syphilis have been found in Papua New Guinea, and in fact the first findings in a new epidemic were the clinical cases.[10]

Similarly, in New Caledonia, there has been a large increase in the number of reported cases of both syphilis in all stages (since serological testing became more widely available in 1975) and early syphilis (table VII).

TABLE VII *Syphilis in New Caledonia (cases of early syphilis given as percentage of total)*

Year	*Total No of cases*	*Cases of early syphilis* *No*	*% of total*	*Other cases*
1955	78	36	46·2	42
1966	85	1	1·2	84
1970	5	5	100·0	0
1971	12	3	25·0	9
1975	655	310	47·3	345
1976	783	280	35·8	503

Of the 761 cases of syphilis seen at the Dermatovenereological Dispensary in Noumea between 1976 and 1977, 168 (22·1%) were clinical cases. The ratio of clinical cases to the total number may be expected to fall as screening techniques improve and control is established.

The three patients with syphilis (table VI) in Tonga (one male Tongan soldier and two female Tongan prostitutes) had primary syphilis, which was confirmed by both laboratory and serological investigations.[7] By 1977 cases of early congenital syphilis were being observed with increasing frequency in both New Caledonia and Fiji.[11]

There is no doubt that the phenomenon of emergent syphilis is one of increasing importance, particularly as 12 of the 20 island territories have yet to report a single case.

PREVALENCE OF YAWS

Yaws was highly prevalent throughout the Pacific Islands in earlier years. In 1933, for example, there were only 15 cases of venereal disease in Fiji and 2249 cases of yaws; in 1937, 4500 cases were reported.[12 13] The horrifying effects of yaws in Guam have been recorded by Butler.[14]

Today yaws has apparently been eradicated in many islands but it has not yet completely disappeared; in recent years some cases have been found in the western region—particularly in Papua New Guinea, which accounted for all but three of the 883 reported cases in 1978 (table VIII).

TABLE VIII *Yaws in the South Pacific*[5-8]

Region	*No of cases†* 1973	1974	1977	1978	*Rate** *(1978)*
Papua New Guinea	954	504	930	880	29
New Hebrides	0	3	23	3	3
Fiji	0	1	0	0	0
Solomons	1	0	0	0	0
Tonga	14	3	0	0	0
Trust Territory Pacific Islands	1	0	0	0	0
Total No of cases	970	511	953	883	
Rates per 100 000	23	12	20	18	

*Per 100 000 population
†Nil returns from American Samoa, Fiji, French Polynesia, Gilbert Islands, Guam, Nauru, Niue, Norfolk Island, Pitcairn Island, Tokelau, Tuvalu, Wallis and Futuna, and Western Samoa

In the smaller islands, the improvement in communications, the expanding tourist industry, and the increased number of schools for the local children seem to have led to the disappearance of yaws; if these conditions are maintained the disease is unlikely to return.

An epidemic of tropical ulcer in the Cook Islands in 1976 caused great concern and resulted in the reporting of 276 cases of so-called yaws in 1976 and 463 more in 1978.[7 8] These cases were originally returned as yaws but on investigation the diagnosis was not supported by pathological findings and they have been excluded from table VIII.

Conclusion

All data relating to the sexually transmitted diseases in the South Pacific as a whole are dominated by those from Papua New Guinea. These will therefore be considered in more detail in the third and last paper in this series.[16]

Particular thanks are expressed to the Secretary General E Macu Salato of the South Pacific Commission for permission to use the maps (which have been modified) and to this organisation, through Dr T Kuberski, and also to Dr G Causse of the World Health Organisation, Geneva, for kindly providing statistical material, and to Dr G Bernard of the Dermatovenereological Dispensary, Noumea, New Caledonia, for his demonstration of clinical material.

References

1. Willcox RR. Venereal diseases in the islands of the North Pacific. *Br J Vener Dis* 1980;**56**:173-7.
2. Zimmet P. Diabetes in Pacific populations—a price for westernisation. In: *Proceedings of the Sixth Asia and Oceania Congress of Endocrinology* (Singapore, January 1978), 1978:256-65.
3. World Health Organisation. Gonococcal infections. *Wld Hlth Stat Rep* 1975;**28**:118, 125.
5. South Pacific Commission. *Annual Summary of the 1973 Infectious Disease Reports*, Noumea, New Caledonia: South Pacific Commission, 1974.
6. South Pacific Commission. *Annual Summary of the Reported Diseases for 1974*, Noumea, New Caledonia: South Pacific Commission, 1975.
7. South Pacific Commission. Reported cases of notifiable diseases for the South Pacific region countries. *Annual Report for the Year 1977*, 1978.
8. South Pacific Commission. Reported cases of notifiable diseases for the South Pacific countries. *Annual Report for the year 1978*, Noumea, New Caledonia: South Pacific Commission, 1979.
9. Willcox RR. The current status of the sexually transmitted diseases with special reference to the South Pacific Region. *Report on Regional Workshop on Cholera and other Epidemic Diseases in the Pacific, 24-28 April 1978*. Noumea, New Caledonia: South Pacific Commission, 1978;63-6.
10. Rhodes FA, Anderson SLJ. An outbreak of treponematosis in the Eastern Highlands of New Guinea. *Papua New Guinea Med J* 1970;**13**:49-52.
11. Kubenski T. Sexually transmitted diseases in the South Pacific region. *Report on Regional Workshop on Cholera and other Epidemic Diseases in the Pacific, 24-28 April 1978*. Noumea, New Caledonia: South Pacific Commission, 1978;67-8.
12. *Fiji Annual Med Hlth Rep Suva*, 1933.
13. *Fiji Annual Med Hlth Rep Suva*, 1937.
14. Butler CS. *Syphilis Sive Morbus Humanus*, Lancaster, Pennsylavania: Science Press, 1936.
15. *Official Airline Guide*, Hinsdale, Ill, USA: Reuben H Donnelley Corp, 1978.
16. Willcox RR. Venereal disease in the Pacific Islands. III Papua New Guinea. *Br J Vener Dis* 1980 (in press).

Br J Vener Dis 1980;**56**:210-7.

Immunoregulatory properties of serum from patients with different stages of syphilis

J J THOMPSON, R J MANGI, R LEE, AND J M DWYER
From the Division of Immunology, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut, USA

SUMMARY The response of lymphocytes from 17 patients with primary, secondary, and tertiary syphilis to phytohaemagglutinin (PHA) and to allogeneic lymphocytes was normal in heterologous serum; however, the responsiveness of cells from some patients with primary and secondary disease was significantly reduced in the presence of autologous serum. As cells from healthy controls invariably responded better to these stimuli in autologous serum, the sera from 81 patients with syphilis were screened for immunosuppressive properties. Sera from 25 primary, 32 secondary, two tertiary, six congenital, and 16 latent cases of syphilis were examined for their ability to reduce the responsiveness of normal cells to PHA. These experiments were performed with test sera as the sole source of serum for the cultures or with test sera added to cultures containing optimally supportive amounts of pooled human plasma.

Stimulation of normal cells from one control in human plasma and 20% test serum showed that only in sera from congenital cases of syphilis was the mean response significantly different from the response seen in control sera; a significant increase in the response to stimulation occurred. The range of response to PHA with sera from cases of primary and secondary syphilis was wider than with normal sera. Sera from five (20%) cases of primary and 14 (44%) cases of secondary syphilis appeared to be immunosuppressive. When retested on another sample of normal cells, these sera were consistently immunosuppressive even in the presence of 15% pooled human plasma. Thus, in early syphilis antigenic stimulation may result in the release from suppressor cells of non-specific immunoregulators of cell-mediated immunity. Such phenomena may be a prelude to the development of tolerance to treponemal antigens. In congenital syphilis the development of suppressor cells may be impaired, resulting in the apparent immunostimulatory properties of serum from such cases.

Introduction

Many biological systems are regulated by balancing opposing forces. Conclusive evidence is now available to extend this principle to the immune response.[1] Reaction to foreign antigens represents, at any given moment, a balance between immunoregulatory and suppressive forces, provided mainly by a subset of thymus-derived lymphocytes and effector mechanisms that produce inflammatory responses by antibody production or cell-mediated immunity (CMI).[2 3] Although triggered by specific antigen, suppressor influences are frequently non-specific and can thus produce generalised immunosuppression. In a number of granulomatous diseases and parasitic infections interaction between the host's immune system and the causative antigen(s) produces a response that favours suppression rather than effector mechanisms.[4-6] This gives an invading organism capable of preferentially activating suppressor machanisms definite survival advantages. The immunological response to the micro-organism causing syphilis may be similar. An adequate cell-mediated immune response to *Treponema pallidum* may be hampered by specific activation by the organism of suppressor mechanisms.[7 8] Consistent with this concept are studies which suggest that in active disease both delayed hypersensitivity and in-vitro lymphocyte responsiveness to *T pallidum* are best seen after treatment.[9-11]

Address for reprints: Dr J M Dwyer, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, USA

Received for publication 13 August 1979

The suppression of lymphocyte responses to mitogens and non-treponemal antigens in syphilis seems definite, although conflicting data exist on the degree and time of appearance of this non-specific immunosuppression. In two studies lymphocyte responsiveness to phytohaemagglutinin (PHA), a T-cell mitogen, was not reduced at any stage of the disease.[12 13] However, most studies show suggestive if inconclusive evidence for altered CMI in primary, secondary, and congenital syphilis. In a retrospective study of the histology from the spleen of children who died of congenital syphilis before the age of 3 numbers of lymphocytes in T-cell areas were decreased.[8] Inguinal node biopsies from some untreated patients with secondary disease showed a similar depletion.[8] Using cells from subjects with primary, secondary, tertiary, and latent syphilis, other workers[8 9] concluded that the lymphocytes from patients with primary and secondary disease responded poorly when stimulated by PHA in the presence of their own plasma. Furthermore, the plasma from patients with secondary disease could reduce the response to PHA of cells from a healthy subject. Some authors have suggested that the immunosuppressive factors could only be demonstrated with plasma.

If active suppression of the host's response to *T pallidum* is accomplished by antigen-specific activation that in turn leads to the production of non-antigen specific suppressor lymphokines, these humoral agents will be concentrated at infection sites. Thus, remote from such sites biological fluids (for example, plasma) may contain diluted lymphokines capable of inducing a weak but measurable non-specific suppression of lymphocyte function.

We have examined plasma samples from patients with syphilis for immunosuppressive properties, but previous studies from this laboratory of a similar question in sarcoidosis[14] have shown that it is difficult to prove that serum factors are actively immunosuppressive rather than passively non-supportive. In the present study stimulation by PHA of lymphocytes from one healthy subject in many different but normal sera produced an extremely wide variation (100-fold) in the degree of stimulation measured. We therefore consider that biologically significant suppressors in sera should be looked for by adding test sera to cultures already with optimal supportive conditions incorporating pooled human plasma. We have used this approach in an attempt to clarify and extend the significance of the previous studies of serum modifiers of cell-mediated immunity in syphilis. Furthermore, we have looked at the immunocompetence of thoroughly washed lymphocytes from patients with syphilis by stimulating such cells with PHA and allogeneic lymphocytes (mixed lymphocyte reaction, MLR) in media containing normal sera.

Materials and methods

STUDY POPULATION

Cells

To assess cellular immune competence in syphilis, cells from 17 patients and 10 controls were studied. Five primary, 11 secondary, and one tertiary case of syphilis were evaluated. The patients attended either the Mothaven or Morisanea Clinics of the New York City Health Department or the Yale-New Haven Hospital. The 10 controls were laboratory personnel with negative results to the Venereal Disease Research Laboratory (VDRL) test. Only patients with ulcers giving positive results on darkground microscopy were considered to be cases of primary syphilis. Clinical signs of secondary disease, consisting of a rash as well as a VDRL titre consistent with active secondary disease, were required for a diagnosis of secondary syphilis. The one late case had untreated tabes dorsalis. The clinical history, laboratory investigations, treatment, age, sex, and race of each case were recorded.

Sera

To assess humoral immunoregulators in syphilis, sera from 81 patients and 33 controls were examined for the presence of immunosuppressive factors. Sera from 25 primary, 32 secondary, two late, six early congenital, and 16 latent cases of syphilis were studied. Most of these sera, as well as the age, sex, race, serological results, and history of the patients from whom they were drawn, were supplied by the Communicable Disease Centre (Atlanta, Georgia, USA).

Lymphocyte cultures

Heparinised blood was allowed to sediment for two hours at 37°C. The leucocyte-rich plasma was centrifuged twice at 1800 rev/min at 4°C for 10 minutes. The pellet was resuspended in 2 ml of Roswell Park Memorial Institute medium 1640 (RPMI 1640) containing penicillin, streptomycin, L-glutamine, and heparin.[14] The cell concentration was adjusted to 4×10^6 per ml, and 0·05 ml ($0{\cdot}2\times10^6$ cells) was placed in a microtissue culture well. For studies of cellular competence, stimulation with PHA and allogeneic lymphocytes (MLR) were performed with the cells in both pooled human plasma (PHP) 15% and 15% PHP with 20% autologous plasma (AP) added. To assess the effect of serum from patients with syphilis on normal cells, the latter were stimulated with PHA in media containing either 20% test serum or 15% normal PHP as well as 20% test serum. These decisions were taken after initial studies (reported below) showed wide variations in the responsiveness of normal cells in various normal sera. Cells were stimulated by an optimal concentration of PHA (1/250 stock solution) or a mixture of inactivated lymphocytes containing equal numbers of cells from four subjects (total $0 \cdot 4 \times 10^6$). Cultures using PHA were pulsed with tritiated thymidine and terminated after three days whereas the MLRs were pulsed and terminated after seven days. Samples were harvested using a multiple automated sample harvester and thymidine uptake measured by a standard method.[14] Mean values were obtained from quadruplicate studies. Increases in absolute counts per minute (cpm) after stimulation were calculated by subtracting from the counts obtained from stimulated cultures the baseline cpm emitted from unstimulated cells.

STATISTICAL METHODS

Statistical significance for the differences in lymphocyte responsiveness was obtained by comparing mean values and the standard error of the mean (SEM) using Student's *t* test.

Results

EFFECTS OF NORMAL PLASMA ON RESPONSE OF NORMAL LYMPHOCYTES TO PHA

The results of studies in which cell cultures from the one individual were established on one day and stimulated with one dose of PHA are given in fig 1. The cultural conditions were identical except for the plasma added to the culture media. Most striking is the effect of heterologous plasma (HP) on responsiveness to PHA. Ten different normal plasma samples produced widely scattered results with a mean ±1 standard deviation (SD) for absolute cpm of 27 753±14 132. This can be compared to the mean cpm ±1 SD of 10 cultures containing the same cells cultured in autologous plasma (AP), 37 675±2 875, or 10 samples of pooled human plasma (at least four separate samples pooled) of 36 280±3864. Clearly, some of the normal HP samples looked immunosuppressive but were simply non-supportive. In a clinical study, four of the 10 samples would have been considered abnormal. By culturing these same cells in 15% PHP and adding 20% AP or HP, normal stimulation with an acceptable standard deviation was obtained. For this reason we considered that biologically significant suppression of lymphocyte responsiveness by syphilitic plasma should be looked for in cultures already optimally supported by pooled human plasma.

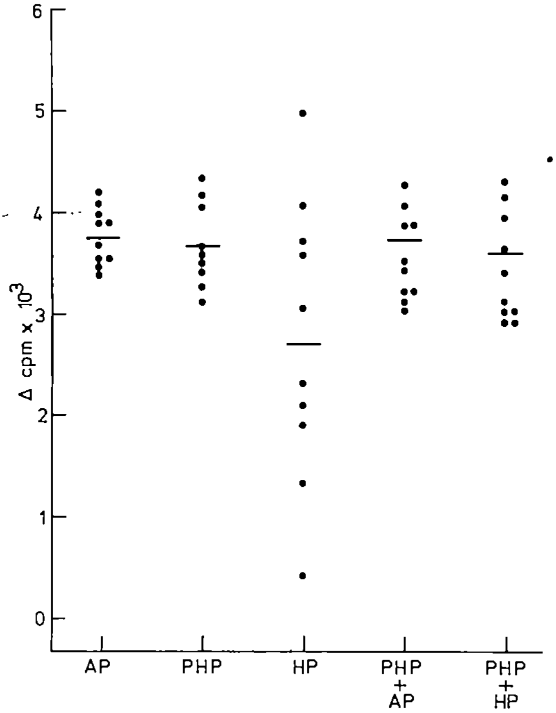


FIG 1 *Results of 10 identical cultures established with cells from one individual in his own plasma (15%). The variation in DNA synthesis so obtained (which is inherent in the methodology used) was compared to the variation obtained when different plasma samples were substituted for AP. The same cells were stimulated in 10 different samples of PHP (15%), HP 15%, 15% PHP and 20% AP, and 15% PHP and 20% HP.*

STIMULATION OF LYMPHOCYTES FROM SYPHILITIC PATIENTS

Phytohaemagglutinin

The data obtained from stimulating normal cells and cells from patients with primary, secondary, and tertiary syphilis with PHA in 15% PHP, or in 15% PHP and 20% AP, are shown in fig 2 together with the mean increase in absolute cpm and a range of cpm representing 2 SD. The response of normal control cells to PHA was better in media containing AP ($p < 0 \cdot 001$); the same was true for most patients with syphilis. However, the cells from three primary and four secondary cases of syphilis responded better in PHP suggesting a positive inhibitory effect of their serum on these cells. There was no significant difference between the baseline counts from unstimulated cultures in either PHP or AP. Although the mean response to PHA of the cells from patients with secondary syphilis was lower than that of the corresponding control group, the

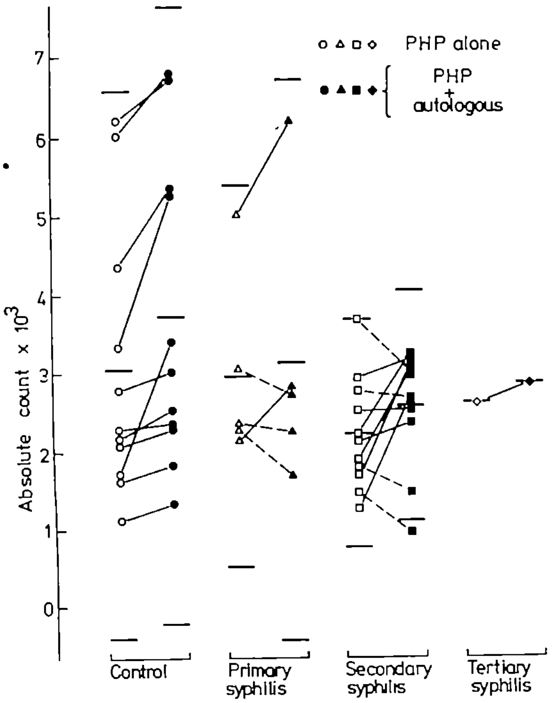


FIG 2 *Results of stimulation of normal cells and cells from patients with syphilis with PHA in either 15% PHP or 15% PHP and 20% AP. The mean increase in cpm±2 SD is given for each group.*

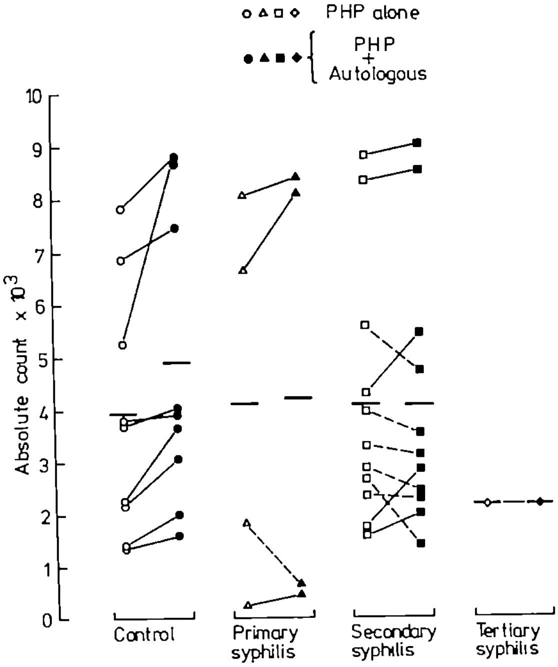


FIG 3 *Results of stimulation of cells from controls and patients with syphilis with allogeneic cells in either PHP or PHP and AP.*

differences were not statistically significant. Thus, no evidence was found to suggest that on group analysis the cells from patients with primary, secondary, or tertiary disease in the absence of AP were less responsive to PHA than normal cells (fig 2).

Pooled irradiated lymphocytes

The results obtained using pooled irradiated lymphocytes paralleled those found with PHA (fig 3). Again, normal cells responded better to allogeneic lymphocytes when AP was present ($P<0 \cdot 05$). Cells from one of the four patients with primary syphilis responded better in PHP cultures. Cells from six of 11 subjects with secondary disease responded less well to allogeneic lymphocytes in the presence of AP. Despite this clear evidence of some immunosuppressive factor in the plasma of some patients with syphilis, no intrinsic defect in the ability of the cells to respond to allogeneic lymphocytes was noted.

RESPONSE OF NORMAL CELLS TO PHA

Effect of syphilitic sera

Initially, the response of one control's normal cells to PHA was studied in 15% PHP containing 20% test serum. Thirty-two control sera and sera from 24 primary, 26 secondary, six congenital, and 13 latent cases of syphilis were tested (first series). All cultures were set up on the same day thus avoiding the inherent variation in the response of cells from one individual at different times. Comparison of the mean response to PHA and the standard deviation observed in each group by unpaired *t* tests showed that only in the presence of the sera from congenital cases was there a significant difference. Compared with the response of normal cells in control sera and PHP, the same cells in the presence of PHP and sera from congenital cases showed a significant increase ($P<0 \cdot 001$) in their response to stimulation (fig 4). The response to PHA of the cells from this control subject did not vary significantly when a range of 10–40% PHP was tested to ensure that the presence of 35% plasma/serum in the cultures was not in itself a stimulating or suppressive factor. As shown in fig 4, the mean response of normal cells in sera from primary or secondary cases did not differ significantly from that when control sera were used. However, the range of response was different. With sera from four cases of secondary disease and one case of primary disease, the stimulation observed fell more than 2 SD from the mean value observed with

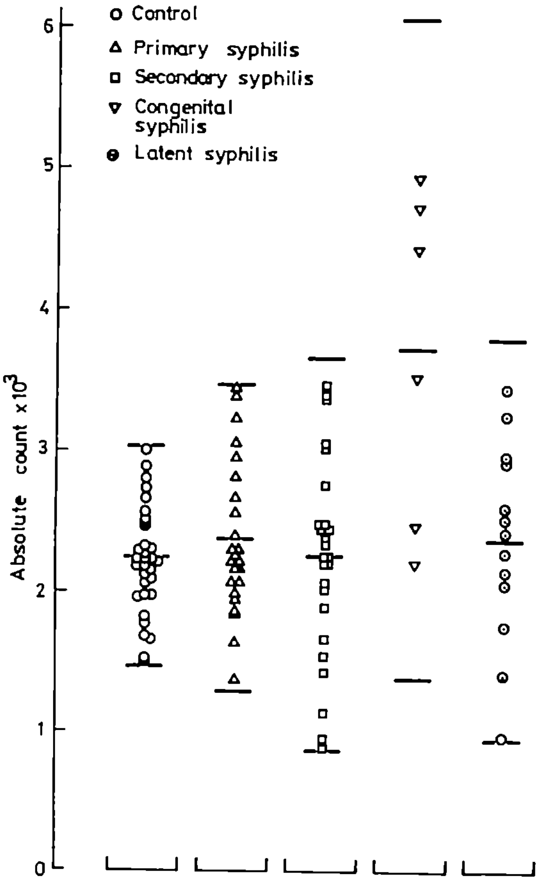


FIG 4 *Results of stimulation of lymphocytes from one control with PHA in 15% PHP containing 20% test sera.*

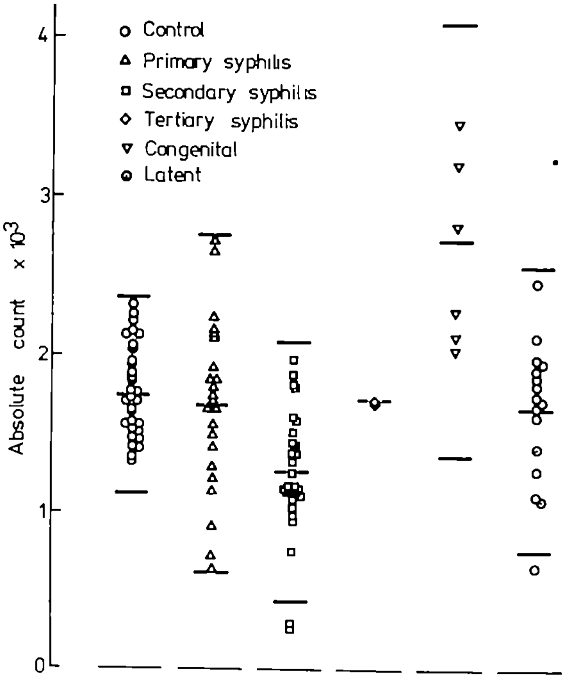


FIG 5 *Results of stimulation of lymphocytes from one control with PHA in 20% test sera without PHP.*

control sera. Because of this observation, and the suggestions from studies using the patients' cells that some sera from cases of primary or secondary disease were immunosuppressive, a second study was performed comparing the cellular response of normal cells to PHA in test sera alone and in test sera with PHP.

Effect of test sera with and without PHP

The effects of sera from 32 controls and 24 primary, 27 secondary, two late, six congenital, and 16 latent cases of syphilis on the response of normal cells to PHA were evaluated. The test sera were used either as the sole serum source (20%) (fig 5) or together with 15% PHP (fig 6). One healthy control supplied all the cells for this study and cell cultures were set up on the same day. With the exception of cultures containing sera from cases of congenital syphilis, the mean of the stimulation observed was higher when PHP was added to test sera, even in the studies using normal sera. Clearly, PHP is able to dilute the immunosuppressive effect that some normal and test sera exert on normal cells (fig 6). Comparison of the mean and standard deviation of the stimulation observed in studies where PHP was added to test sera again showed no significant difference between the response of the normal cells to PHA in control sera or sera from cases of primary, secondary, or latent syphilis. Again, the exception was in sera from congenital cases, which increased the degree of stimulation. No significant difference between the mean stimulation values obtained with control sera alone and with sera from primary, late, or latent cases of syphilis alone was noted. However, when normal cells were cultured in serum from secondary cases of syphilis without added PHP the response to PHA was significantly reduced ($p<0 \cdot 05$) (fig 5). The use of sera from congenital cases of syphilis without PHP was associated with significantly better responses to PHA than when normal sera were used ($p<0 \cdot 001$). A comparison of the range of response in the different conditions shows a wider scatter with sera from cases of primary and secondary syphilis than with normal sera.

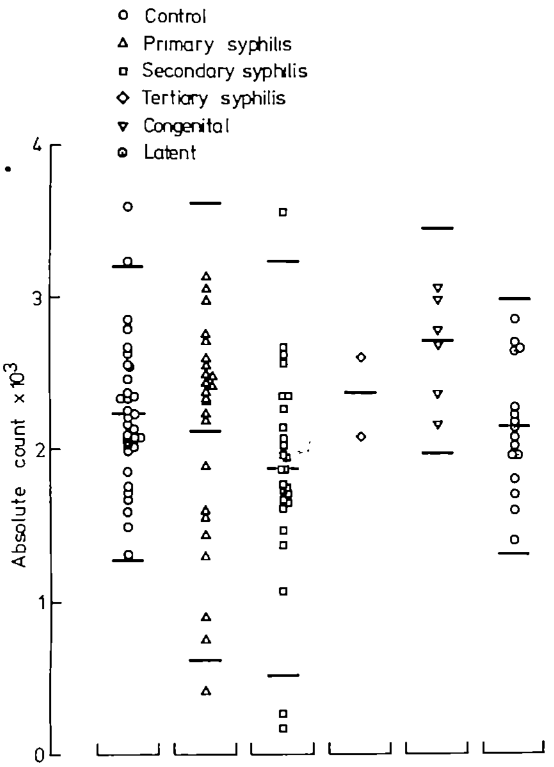


FIG 6 *Results of stimulation of lymphocytes from one control with PHA in test sera containing 15% PHP.*

REPRODUCIBLE IMMUNOSUPPRESSION BY SELECTED SYPHILITIC SERA

Sera from five cases of primary and 14 cases of secondary syphilis, previously associated with levels of stimulation of normal cells that fell 2 SD below the mean value observed when the same cells were cultured in normal sera, were retested together with 13 control sera. Normal cells from a healthy control, not previously used in these experiments, were stimulated with PHA in the presence of 20% test sera and 15% PHP. With these selected sera the presence of PHP did not dilute their immunosuppressive effect. When the mean increase in absolute counts emitted by the normal cells after PHA stimulation in normal sera are compared with the values obtained with sera from primary and secondary cases of syphilis a significant reduction ($P<0\cdot05$ and $P<0\cdot001$ respectively) is observed in the presence of these latter sera. As the cells had an optimal nutritional supply provided by the PHP these selected sera appear to be significantly and reproducibly immunosuppressive.

Discussion

Shortly after the organism causing syphilis was discovered in 1905,[15] studies to determine the degree of immunity from reinfection that followed primary exposure to *T pallidum* began. These studies have been inconclusive. Perhaps as a result of poor antigenic preparations, numerous attempts to elicit delayed hypersensitivity (DH) reactions to treponemal antigens have provided inconsistent data.[16-21] The available data suggest that DH to treponemal antigens is absent in the primary and secondary stages of the disease whereas some DH is occasionally seen in later stages of the disease and in congenital disease.

Better evidence for the development of immunity to syphilis came from a study in which virulent organisms were inoculated into volunteers whose subsequent clinical and serological progress was carefully monitored.[22] The study concluded that early treatment of syphilis probably prevented the development of any immunity to the organism, but that pretreatment with heat-killed organisms significantly reduced subsequent reinfection with live organisms. Such an anamnestic response could not be correlated to titres of antibody against *T pallidum* and seemed to relate more closely to cell-mediated immune functioning.

Recently, emphasis has been placed on the in-vitro response of lymphocytes to improved preparations of syphilitic antigens.[12 13 23-26] Most of these studies measured a lymphoblastic response by morphological criteria rather than by measuring DNA synthesis; however, in general they agree that sensitised lymphocytes are present and can respond to syphilitic antigens in late primary disease and persist from that time. These studies require confirmation with more sensitive techniques.

If we can accept that most subjects can develop a cellular immune response to these antigens, clearly the clinical course of some patients with syphilis suggests that an inadequate immune response to *T pallidum* can occur early in the disease. Our data do not suggest that the lymphocytes from patients with primary or secondary disease respond abnormally to either PHA or allogeneic lymphocytes. Clearly, however, sera from some primary and many secondary cases of syphilis are capable of reducing the response of these cells and normal cells to both PHA and allogeneic lymphocytes. It remains to be shown that the presence of these factors in vivo is associated with a less favourable clinical course.

Previous data from this laboratory[14] have pointed out the difficulty of assessing immunosuppressive properties of individual sera. Normal lymphocytes respond reliably to both PHA and allogeneic lymphocytes in autologous serum but the variation of response in normal but heterologous sera exceeds two logs. This range can be considerably reduced by using pooled human plasma. From the present studies we suggest that an active immunosuppressive property of a serum sample, as opposed to a passive non-supportive property, can only be proved when cells in culture are supplied with optimal amounts of pooled human plasma before the test serum is added. A suppression of the response in these circumstances is likely to be biologically, although not necessarily clinically, significant.

Although it is difficult to assign a degree of responsiveness to PHA and allogeneic lymphocytes that correlates with adequate in-vivo CMI function, it seems likely that the degree of real suppression noted here would still leave most lymphocytes responding within the lower limits of response encountered in healthy individuals. A weak immunosuppressive effect of serum could result from dilution in the plasma volume of an agent exerting a much more powerful effect at the tissue level. The effect of serum from cases of primary and secondary disease on the responsiveness of sensitised lymphocytes to treponemal antigens would provide more relevant information but would require a more sensitive in-vitro assay than has so far been described. It has been suggested[27] and shown[28] that the presentation of large amounts of antigen to sensitised lymphocytes can produce a transient, generalised suppression of CMI; this suppression ends once high-zone tolerance, specific for the desensitising antigen, develops. Such a non-specific humoral immunosuppressive factor may be the product of suppressor T cells.[27] In patients with late primary and early secondary disease, when numerous foci of infection are present, the conditions necessary for eliciting such factors—which could be detected in serum by the methods detailed here—may exist. Perhaps a progression to true immunological tolerance to treponemal antigens occurs in many cases of late syphilis. Once tolerance developed, non-specific humoral immunosuppressive factors may disappear.

The immunostimulatory nature of the sera from cases of congenital syphilis is definite but unexplained. In view of the histological data showing decreased numbers of lymphocytes in the spleen of children with this disease[8] and the fact that the spleen is a major source of suppressor T cells, it seems reasonable to suggest that congenital infection may lead to a lack of suppressor T cells and so to a lack of the humoral regulation of CMI responses which they usually provide. Sera containing helper factors but not regulatory factors would appear to be immunostimulatory. If this explanation was correct, then other phenomena associated with defective T-cell regulatory functions—such as the incidence of autoimmune reactions—would be increased in such patients. The literature contains no information on this matter. Clinical pressures demand more research into these questions; with improved technology, we are studying separated effector and suppressor T cells in humans.

Support for this work was given by NIH training grant 00271 and NIH grant AI-11785.

References

1. Bullock WW, Katz DH, Benacerraf B. Induction of T lymphocyte responses to a small molecular weight antigen. III T-T interaction to determinants linked together: suppression *vs* enhancement. *J Exp Med* 1975;**142:**261-74.
2. Feigheny C, Whelan CA, Weir DG, Greally JF. In-vitro studies of suppressor cell function in human peripheral blood mononuclear cells. *Clin Exp Immunol* 1978;**32:**459-65.
3. Gershon RK. A disquisition on suppressor T cells. *Transplant Rev* 1975;**26:**170-85.
4. Dwyer JM, Mangi RJ, Gee B, Kantor FS. Comparison of the anergy of sarcoidosis with experimentally induced anergy in guinea pigs. *Annals NY Acad Sci* 1976;**278:**29-35.
5. Hillinger SM, Herzig GP. Impaired cell-mediated immunity in Hodgkin's disease mediated by suppressor lymphocytes and monocytes. *J Clin Invest* 1977;**59:**1620-7.
6. Jayawardena AN, Waksman BH. Suppressor cells in experimental trypanosomiasis. *Nature* 1977;**265:**539-41.
7. Levene GM, Wright DJM, Turk JL. Cell-mediated immunity and lymphocyte transformation in syphilis. *Proc Royal Soc of Med* 1971;**64:**14-8.
8. Levene GM, Turk JL, Wright DJM, Grimble AGS. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. *Lancet* 1969;ii:246-7.
9. From E, Thestrup-Pedersen K, Thulin H. Reactivity of lymphocytes from patients with syphilis towards *T pallidum* antigen in the leucocyte migration and lymphocyte transformation tests. *Br J Vener Dis* 1976;**52:**224-9.
10. Wright DJM, Grimble AGS. Why is the infectious stage of syphilis prolonged? *Br J Vener Dis* 1974;**50:**45-9.
11. Musher DM, Schell RF, Jones RH, Jones AM. Lymphocyte transformation in syphilis: an in-vitro correlate of immune suppression in vivo? *Infect Immun* 1975;**11:**1261-4.
12. Badanoiu A, Gavrilesco M, Nicolau G, Circiumaresco T. Lymphoblastogenese immunoallergique et antigène treponemique in vitro dans la syphilis. *Roum Path Exp Microbiol* 1969;**28:**1901-6.
13. Janot C, Grandidier M, Pupil P, Thomas J-L, Beurey J, de Lavergne E. Le test de transformation lymphoblastique au cours de la syphilis. *Presse Medicale* 1971;**79:**1901-4.
14. Mangi RJ, Dwyer JM, Kantor FS. The effect of plasma upon lymphocyte response in vitro. Demonstration of a humoral inhibitor in patients with sarcoidosis. *Clin Exp Immunol* 1974;**18:**519-28.
15. Schaudinn F, Hoffman E. *Arb Kais Gesund* 1905;**22:**528.
16. Neisser A. *Arb Kais Gesund,* 1911; **37:**201.
17. Noguchi, H. *J Exp Med* 1911;**14:**557.
18. Chesney, AM. Immunity in syphilis. *Medicine Monographs XII* Baltimore; Williams and Wilkins, 1972.
19. Marshak LC, Rothman S. Skin testing with a purified suspension of *T pallidum. Amer J Syphilis* 1951;**35:**35-41.
20. Thivolet J, Simeray A, Rolland M, Challut F. Etude de l'intradermoreaction aux suspensions de treponèmes formolées (Souche Nichols Pathogene) chez les syphilitiques et les sujets normaux. *Annales de l'Institut Pasteur* 1953;**83:**23-33.
21. Laird SM, Thorburn AL. Assessment of the "Luotest" in late syphilis. *Br J Vener Dis* 1966;**42:**119-21.
22. Magnuson HJ, Thomas EW, Olansky S, Kaplan BJ, DeMello L, Cutler JC. Inoculation syphilis in human volunteers. *Medicine* 1956;**35:**33-82.
23. Chieregato GC, Faldarini G, Pagnes P. La stimolazione linfocitaria. In vitro in sogetti luetici. *Minerva Dermatol* 1967;**42:**406-7.

24. Dwyer JM, Mackay IR. Modern concepts of antigen binding receptors on lymphocytes. Their nature and role in immune responses. *Rev Europa Etudes Clinique et Biol* 1971; **16**: 743-6.
25. Beurey J, Weber M, Janot C, Grandidier M, Pupil P. L'allergie au cours de la syphilis. Interet du test de transformation lymphoblastique. *Ann Dermatol Syphilol* 1971; **98**: 536-8.
26. Basset A, Badanoiu A, Grosshans E, *et al.* Studio sulla reattivita immuno-cellare nella lue con il test della transformazione blastica dei linfociti in vitro (TTL). *Minerva Medica* 1972; **63**: 3031-6.
27. Gershon RK. T-cell control of antibody production. *Contemporary Topics in Immunobiology.* Vol 3, chap 1, New York: Plenum Press, 1974.
28. Dwyer JM, Kantor FS. In-vivo suppression of delayed hypersensitivity: Prolongation of desensitization in guinea pigs. *J Exp Med* 1975; **142**: 588-99.

Br J Vener Dis 1980;**56**:218-20.

Immunoglobulin-bearing polymorphonuclear leucocytes in primary syphilis

J D BOS, F HAMERLINCK, AND R H CORMANE
From the Department of Dermatology, University of Amsterdam, Amsterdam, the Netherlands

SUMMARY Non-specific defence mechanisms are thought to be ineffective in eliminating *Treponema pallidum* after infection in the early stages, but studies aimed at the elucidation of this phenomenon are few.

In this study, the number of IgG-bearing polymorphonuclear leucocytes in patients with primary syphilis was increased; this is most probably a normal phenomenon of infection.

Introduction

The non-specific defence mechanisms are generally thought to be ineffective in eliminating *Treponema pallidum* after infection has been established. The combined efforts of polymorphonuclear leucocytes, mononuclear macrophages, and non-specific humoral factors are unable to neutralise the invading micro-organisms in the early stages of syphilis. Studies of polymorphonuclear leucocytes (PMNL) in syphilis are few. Histologically, PMNL are seen in the initial stage of primary syphilis, accumulating in the perilymphatic spaces, since later they are replaced by lymphocytes and plasma cells.[1]

Recently the elimination of treponemes by phagocytes after 7-8 hours of penicillin therapy could be demonstrated in early syphilitic lesions in humans using the electron microscope.[2] In another study, syphilitic infection of rabbits was found to be significantly associated with increased metabolic activity of phagocytes as detected with the nitroblue tetrazolium test.[3]

In this study, the number of immunoglobulin-bearing PMNL, isolated from peripheral blood, in a group of patients with primary syphilis was determined. Variations in the percentages of different Ig classes present on the membrane of circulating PMNL have previously been found in diseases such as allergic contact dermatitis and psoriasis.[4]

Patients and methods

STUDY POPULATION

The patients in this study were referred by the outpatient department of our hospital. A total of seven patients, all with primary syphilis, were studied. This group of patients was the same as that described in two previous papers on the percentages of B and T lymphoid cells in these patients.[5 6] All the patients were male; five were untreated at the time of investigation whereas two were treated for less than one week (patients 9 and 12). The time interval from date of infection to time of investigation varied from 4-12 weeks.

HEALTHY CONTROLS

Ten healthy staff members served as controls for the quantitative determination of immunoglobulin-bearing PMNL. All were male and of the same age group as the patients studied.

ISOLATION OF POLYMORPHONUCLEAR LEUCOCYTES

Separation of PMNL was carried out according to the method of Böyum[7] with some minor modifications as described below. Fifty millilitres of peripheral blood were collected aseptically in Erlenmayer bats and defibrinated by shaking for 10 minutes with 50 glass beads; to this, 20 ml of phosphate-buffered saline (PBS) was added. This suspension was layered on top of 40 ml Isopaque-Dextran* for 45 minutes; the red cells are clumped at

Address for reprints: Dr J D Bos, Academisch Ziekenhuis, Grimburgwal 10, 1012 GA Amsterdam, the Netherlands

Received for publication 4 December 1979

---

*Ten parts Isopaque solution mixed with 24 parts of 6% Dextran (Pharmacia, Uppsala, Sweden).

the interface and sedimented at the bottom of the tube in 1 × *g* gravity field.

The plasma layer was aspirated and washed once in PBS for five minutes at 200 × *g*. The centrifuged cells were resuspended in 80 ml PBS and layered on Isopaque-Ficoll mixture. The latter suspension was centrifuged at 400 × *g* for 20 minutes. The bottom of the tube contained the polymorphonuclear leucocytes.

IN-VITRO IMMUNOFLUORESCENCE STUDIES OF PMNL

After the isolated PMNL had been washed three times in PBS at 200 × *g* for five minutes, the relative percentages of IgG-, IgA-, IgM-, IgD-, and IgE-bearing PMNL were determined by immunofluorescence as described before.[6]

PERIPHERAL BLOOD COUNTS

Total peripheral leucocyte counts and their differentiation were estimated to relate percentage figures to the absolute numbers of PMNL.

STATISTICAL METHOD

The non-parametric Wilcoxon's test[8] with the usual correction for ties, and the normal approximation to obtain the P value, was used for statistical analysis of the numbers of immunoglobulin-bearing PMNL in patients with primary syphilis and in healthy controls.

MATERIALS

The materials used in this study were the same as those used in a previous study on lymphoid cells.[6]

Results

The results of the determination of percentages of immunoglobulin-bearing polymorphonuclear leucocytes for each of the five subclasses of immunoglobulins in patients and controls are given in the table. Absolute numbers of PMNL in the patients with primary syphilis were normal (mean $4\cdot41\times10^9/l$; range $1\cdot39\text{-}8\cdot38\times10^9/l$) and statistical analysis was performed on percentage figures. The exact values of standardised W and two-sided tail probability, resulting from applying Wilcoxon's test to the obtained percentages, are also given in the table.

Although the number of patients studied was small, the percentage of IgG-bearing PMNL was significantly increased in the patients with primary syphilis ($P = 0\cdot0032$). Furthermore, the number of IgE-bearing PMNL were significantly decreased in patients with primary syphilis ($P = 0\cdot0206$) as were the number of IgD-bearing PMNL ($P = 0\cdot0140$).

TABLE *Percentages of immunoglobulin-bearing polymorphonuclear leucocytes in seven patients and 10 controls*

	Immunoglobulin-bearing PMNL (%)				
	IgG	*IgA*	*IgM*	*IgD*	*IgE*
Patient No					
5	5	2	10	1	1
6	15	5	7	5	2
7	3	0	4	2	1
8	11	4	14	2	3
9	11	5	9	3	3
11	5	0	2	0	0
12	4	3	8	0	0
Range	3–15	0–5	2–14	0–5	0–3
Median	5	3	8	2	1
Control No					
1	1·0	2·0	4·0	2·0	1·0
2	1·5	5·0	4·0	7·5	4·0
3	1·5	3·0	7·5	6·0	7·0
4	1·5	0·5	7·3	3·0	1·5
5	1·0	1·3	6·0	5·6	6·3
6	2·4	3·8	7·8	6·5	3·0
7	3·0	2·6	7·5	9·3	10·2
8	6·0	7·0	9·0	7·0	8·0
9	0·5	0·5	2·0	2·0	3·0
10	0·5	0·5	2·5	2·5	2·5
Range	0·5–6·0	0·5–7·0	2·0–9·0	2·0–9·3	1·0–10·2
Median	1·9	2·6	5·8	5·1	4·8
Wilcoxon's test	2·9930	0·0983	1·1761	−2·5070	−2·3640
Probability	0·0032	0·9608	0·2597	0·0140	0·0206

Discussion

In this study, data are presented which suggest alterations in surface immunoglobulins of PMNL as a result of infection with *T pallidum*. The increased number of IgG-bearing PMNL in some but not all patients with primary syphilis may indicate adherence of immune complexes consisting of treponemal antigens and antitreponemal IgG, these complexes being bound by way of C3b and Fc receptors to the membrane of circulating PMNL.[9] Phagocytosis and intracellular processing of treponemal constituents must have taken place since humoral responses were generally present in the patients studied.

Interpretation of the decreased number of IgD- and IgE-bearing PMNL is difficult and its significance too low to allow conclusions to be drawn. Increased numbers of IgG-bearing PMNL do not seem to be disease-specific but seem to be the result of IgG-production as a humoral response to infection. In this study, no data on the ineffectiveness of non-specific defence mechanisms in syphilis are given but the results may indicate the normal processing of immune complexes in primary syphilis. Further studies to elucidate the origin of ineffective non-specific defence mechanisms in syphilis are needed.

References

1. Treponematoses research: report of a WHO scientific group. *Wld Health Org Techn Rep Ser* 1970;**455**:74-8.
2. Wecke J, Bartunek J, Stüttgen G. *Treponema pallidum* in early syphilitic lesions in humans during high-dosage penicillin therapy: an electron microscopical study. *Arch Derm Res* 1976;**257**:1-15.
3. Wicher V, Blakowski, S, Wicher K. Nitroblue tetrazolium test in experimental syphilis. *Br J Vener Dis* 1977;**53**:292-4.
4. Hunyadi J, Hamerlinck F, Cormane RH. Immunoglobulin- and complement-bearing polymorphonuclear leucocytes in allergic contact dermatitis and psoriasis vulgaris. *Br J Derm* 1976;**94**:417-22.
5. Bos JD, Hamerlinck F, Cormane RH. T-lymphoid cells in primary syphilis. *Br J Vener Dis* 1980;**56**:74-6.
6. Bos JD, Hamerlinck F, Cormane RH. Immunoglobulin-bearing lymphoid cells in primary syphilis; quantitative and elution studies. *Br J Vener Dis* 1980;**56**:69-73.
7. Böyum A. Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968;**21**, suppl **97**:1-106.
8. Siegel S. *Nonparametric Statistics.* New York: McGraw-Hill, 1956.
9. Valdimarsson H. Immunity and immunity deficiency. In: Hobart MJ, McConnell I, eds. *The Immune System.* 3rd ed. Oxford: Blackwell Scientific Publications, 1978;317-32.

Br J Vener Dis 1980;**56**:221-2.

Persistent serum immune complexes in syphilis

SIEGFRIED ENGEL AND WOLFGANG DIEZEL

From the Department of Dermatology and Venereology (Charité), Humboldt University, Berlin, East Germany

SUMMARY Serum immune complexes were estimated by a polyethylene glycol precipitation method in 51 patients with early syphilis. The immune complexes were increased in 41% of patients before treatment and decreased to normal limits in only 56% during treatment. The results of the specific antitreponemal tests using dissolved immune complexes show that antibodies from the immune complexes are specific antibodies.

Introduction

The presence of circulating immune complexes (ICs) in early syphilis was first suggested by the evidence of ICs in syphilitic nephropathy.[1 2] Using a radioimmunoassay, Sølling and his co-workers[3 4] also demonstrated circulating ICs in early syphilis and in a case of syphilitic nephropathy. Gamble and Reardan[5] found antitreponemal antibodies in the deposits of ICs by antibody elution tests performed on kidney biopsy material, and Tourville and co-workers[6] demonstrated the presence of treponemal antigen using an indirect fluorescent antibody technique.

In this study, we looked for circulating ICs before and after treatment using a precipitation method and assessed the specificity of circulating ICs.

Patients and methods

Serum samples from 51 local patients with primary (6) and secondary (45) syphilis were investigated before treatment. Of the 43 men (aged 20-71 years), 35 were homosexuals and five were married; of the eight women (aged 18-44 years), four were married.

Serum samples from 38 patients were investigated before and once a week during treatment, the last time being after treatment.

DIAGNOSIS

The diagnosis was established by either darkground microscopy or serological tests—the fluorescent treponemal antibody absorption (FTA-ABS) test, the *Treponema pallidum* immobilisation (TPI) test, the *T pallidum* haemagglutination (TPHA) test, and the Venereal Disease Research Laboratory (VDRL) test.

TREATMENT

Patients were treated with 500 000 units benzylpenicillin twice daily for 15 days and then with either benzathine benzylpenicillin/procaine benzylpenicillin retard 1 200 000 units three times weekly for 15 days or benzylpenicillin 1 megaunit twice daily for a further 15 days (in patients with abnormal cerebrospinal fluid samples).

PRECIPITATION TECHNIQUE

Serum ICs were estimated by the polyethylene glycol (PEG) precipitation method according to the technique of Diezel and co-workers.[7] The principles of the method are precipitation of the ICs in barbital-HCl buffer (pH 8·4) containing 3·5% PEG, sedimentation of the complexes by centrifugation at 12 000 × *g*, dissolution of the precipitates in Biurets reagent, and measurement of the optical density at 550 nm (protein determination).

The mean values determined in 100 healthy controls was 3·87 mg/ml serum (confidence interval $P<0{\cdot}01$).

The ICs used to test the specificity of the antibodies in specific routine antitreponemal tests (the FTA-ABS, the TPI, and the TPHA tests) were washed three times with barbital-HCl buffer containing 3·5% PEG and dissolved in 0·15 mol/l NaCl. The IC solution contained 5 mg protein/ml and was used instead of the patients' sera.

Results

ICs were increased in 21 of the 51 serum specimens obtained before treatment; 20 were from patients

Address for reprints: Dr S Engel, Bereich Medizin (Charité), Humboldt-Universität zu Berlin, Hautklinik und Poliklinik, Schumannstrasse 20/21, 104 Berlin, GDR

Received for publication 10 September 1979

with secondary syphilis and one was from a patient with primary syphilis (table I). The ICs decreased to normal limits during treatment in only about 50% of patients (table II). This phenomenon did not depend on the kind of treatment or the daily dosage of penicillin.

Two specimens from patients with secondary syphilis containing increased amounts of ICs were tested by specific antitreponemal tests; the results were compared with those of two specimens from healthy controls (table III).

TABLE I *Increased immune complexes in patients with primary and secondary syphilis before treatment*

		Increased ICs before treatment	
Diagnosis	*No of patients*	*No*	*%*
Primary syphilis	6	1	16
Secondary syphilis	45	20	44
Total	51	21	41

TABLE II *Increased immune complexes in 38 patients with primary and secondary syphilis before and after treatment*

	Increased ICs	
Diagnosis	*Before treatment*	*After treatment*
Primary syphilis	1	0
Secondary syphilis	15	7
Total	16	7 (44%)

TABLE III *Dissolved immune complexes from patients with secondary syphilis tested by specific antitreponemal tests*

	IC detected by antitreponemal tests		
Specimens	*FTA-ABS*	*TPI (% immobilisation)*	*TPHA (titre)*
Secondary syphilis			
1	+	+/− (36)	+ (1/160)
2	+	+/− (28)	+ (1/160)
Control			
1	−	− (4)	−
2	−	− (0)	−

+ Positive; +/− weakly positive; − negative

Discussion

Using a sensitive precipitation method our investigations have shown that in nearly 50% of patients with secondary syphilis, and in some cases of primary syphilis, pathologically increased amounts of ICs can be found.

Contrary to the findings of Sølling and coworkers[3] the ICs did not decrease to normal limits immediately after treatment. The present incomplete results of the continuing study also indicate that they do not decrease to normal limits a few months after treatment.

The results of the specific serological tests using dissolved ICs show that the antibodies from the ICs are specific antitreponemal antibodies.

Thus it can be concluded that (a) the antigen is a specific antigen from *T pallidum;* and (b) the individual pool of ICs is enlarged by specific antigen antibody complexes. If this conclusion is correct *T pallidum* must still be present in patients after treatment.

There is no doubt that specific antibodies are a constituent of the ICs. The ICs were washed until the supernatant was free of protein, especially of IgG detected by immunodiffusion. Provided that the specific antibodies are detected long after treatment, we consider that the continued serum reactivity after treatment is a consequence of the persisting presence of viable *T pallidum*. This corresponds to the results of several investigators who have found evidence of *T pallidum* in patients after treatment.[8 9]

References

1. Bhorade MS, Carag HB, Lee J, Potter EV, Dunea G. Nephropathy of secondary syphilis: a clinical and pathological spectrum. *JAMA* 1971;**216:**1159-66.
2. Braunstein GD, Lewis EJ, Galvanek EG, Hamilton A, Bell WR. The nephrotic syndrome associated with secondary syphilis: an immune deposit disease. *Am J Med* 1970;**48:**643-8.
3. Sølling J, Sølling K, Jacobsen KU, From E. Circulating immune complexes in syphilis. *Acta Derm Venereol (Stockh)* 1978;**58:**263-7.
4. Sølling J, Sølling K, Jacobsen KU, Olsen S, From E. Circulating immune complexes in syphilitic nephropathy. A case report. *Br J Vener Dis* 1978;**54:**53-6.
5. Gamble CN, Reardan JB. Immunopathogenesis of syphilitic glomerulonephritis. Elution of antitreponemal antibody from glomerular immune complex deposits. *N Engl J Med* 1975;**292:**449-54.
6. Tourville DR, Lawrence HB, Kim DU, Zajd D, Lee J, Reichmann LB, Baskin St. Treponemal antigen in immunopathogenesis of syphilitic glomerulonephritis. *Am J Pathol* 1976;**82:**479-92.
7. Diezel W, Meffert H, Gunther W, Sönnichsen N. Quantitative estimation of serum immune complexes with a simple method. *Dtsch Gesund Wes* 1978;**33:**1822-3.
8. Tramont EC. Persistence of *Treponema pallidum* following penicillin G therapy. *JAMA* 1976;**236:**2206-7.
9. Catterall, RD. Immunität im verlauf der syphilis. *Hautarzt* 1978;**29:**119-26.

Br J Vener Dis 1980;**56**:223-6.

Detection of antigonococcal IgA in cervical secretions by indirect immunofluorescence: an evaluation as a diagnostic test

A McMILLAN,*† G McNEILLAGE,† H YOUNG,‡ AND S S R BAIN†

*From the *Department of Sexually Transmitted Diseases, Black Street, Glasgow, and the †Departments of Venereology and ‡Bacteriology, University of Edinburgh, Edinburgh*

SUMMARY Using an indirect immunofluorescent-antibody method, antigonococcal IgA was detected in the cervical secretions of 56 (72%) of 78 women with untreated gonorrhoea but in only 25 (5%) of 490 non-infected women. The low sensitivity—comparable to that of Gram-stain microscopy—the expense, and the laboriousness of the test militate against its use as a routine diagnostic procedure.

Introduction

Although gonococcal antibodies in the serum of patients with gonorrhoea may be demonstrated by various methods,[1] the detection of these antibodies has not proved useful as a diagnostic test.[2] A serum antibody response may not be apparent until infection has been established for several days, and thus early gonorrhoea may be missed.[2] Serum antibodies, particularly of the IgG class, remain detectable for weeks or months after successful treatment;[3] interpretation of tests based on the demonstration of IgG antibody is therefore difficult. Wilkinson,[4] using a fluorescent-antibody test, demonstrated significantly high titres (>16) of IgM antibody in only 24 (43%) of 55 women with untreated gonorrhoea; Ison and Glynn[5] observed that the detection of IgM antibodies by an enzyme-linked immunosorbent assay was of little diagnostic help.

Gonorrhoea is essentially an infection of mucosal surfaces, and gonococcal antibodies may be demonstrated in the urethral and cervical secretions from infected men and women respectively.[6 7] The local IgA antibody response antedates that of the serum, and in most cases antigonococcal IgA in the secretions cannot be detected two to three weeks after successful treatment.[7]

By microscopical examination of Gram-stained smears of material from the cervix and urethra, only about 55% of women with gonorrhoea will be identified.[8] Culture on selective medium is the most sensitive procedure available for the diagnosis of gonorrhoea in women; however, results are not generally available until 48 hours after the collection of specimens. To control gonococcal infection within a community, a rapid and sensitive method of identifying infected, but asymptomatic, individuals is required. The purpose of this study was to determine if the detection of antibodies in cervical secretions could be used as a diagnostic test for gonorrhoea. Preliminary data are presented in this paper.

Patients and methods

STUDY POPULATION AND DIAGNOSIS

With the exception of pregnant women, all female patients who attended the Department of Sexually Transmitted Diseases, Black Street, Glasgow, and clinics held by one of us (SSRB) at the Department of Venereology, Royal Infirmary, Edinburgh, during a six-month period were studied.

The methods used in both centres for the diagnosis of gonorrhoea have previously been described.[7]

Patients were divided into three groups: (a) 78 women with untreated gonorrhoea; (b) 178 women who had previously been treated for gonorrhoea; and (c) 490 women who had no microbiological evidence of current gonococcal infection or a past history of gonorrhoea.

COLLECTION OF SPECIMENS

Under direct vision, a cottonwool-tipped applicator stick was inserted into the cervical canal to a distance

Address for reprints: Dr A McMillan, Department of Sexually Transmitted Diseases, Black Street, Glasgow G4

Received for publication 28 September 1979

of about 1 cm and allowed to remain in contact with the secretions for about 15 seconds. Secretions were eluted from the swab by placing this in 0·5 ml of phosphate-buffered saline, pH 7·4, containing Tween 20 (0·05% v/v). Samples were frozen at −20°C until required. After thawing and centrifugation at 2000×*g* for 15 minutes, the supernate was used for estimating IgA and IgG concentrations and for the indirect immunofluorescent-antibody test.

ESTIMATION OF IgA AND IgG CONCENTRATIONS

Using commercially available, low-level immunodiffusion plates (Hoescht Pharmaceuticals, Hounslow, Middlesex) the concentrations of IgA and IgG in the supernate of diluted secretions from 18 patients selected at random were determined. Commercially available serum IgG (Travenol Laboratories, Norfolk) was used as reference standard; colostral IgA standard[9] was used in the estimation of secretory IgA.

INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST

The procedure has been described in detail elsewhere.[3] Strain 9 of *Neisseria gonorrhoeae,* as described by O'Reilly and co-workers,[10] was used as antigen. The grading of fluorescence was as follows: 4+, brilliant fluorescence of all organisms; 3+, well defined fluorescence of all organisms in the field; 2+, low density fluorescence of at least 75% of organisms; and 1+, occasional fluorescing organisms. Only a 2+ fluorescence or higher reading was recorded as a positive result.

STATISTICAL METHOD

Statistical comparisons were made using the χ^2 test with Yates's correction.

Results

IMMUNOGLOBULIN CONCENTRATIONS

The mean IgA and IgG concentrations in the supernate of the diluted cervical secretions were $11 \cdot 4 \times 10^{-3}$ g/l (standard deviation, SD $6 \cdot 7 \times 10^{-3}$) and $30 \cdot 5 \times 10^{-3}$ g/l (SD $20 \cdot 6 \times 10^{-3}$) respectively.

INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST

The results obtained when cervical secretions from patients in each of the three groups were examined for IgA and IgG antibodies reactive with *N gonorrhoeae* are given in table I.

IgA

IgA antibody was detected in 56 (72%) of the 78 women with untreated gonorrhoea but in only 25 (5%) of 490 non-infected women; the mean duration of infection in the 22 women in whom antibody was not detected was 16·6 days (range 4–48 days).

Antigonococcal IgA was found in cervical secretions from 24 (22%) of 110 women who had been treated more than 28 days previously (mean interval between treatment and sampling, 422 days).

TABLE I *Results of immunofluorescent-antibody test on cervical secretions from women with untreated and previously treated gonorrhoea and from non-infected patients*

Antibody	*No of patients*						
	With untreated gonorrhoea	*With previously treated gonorrhoea (days between treatment and sampling)*				*Non-infected*	*Total*
		≤7	*8-14*	*15-28*	*≥29*		
IgA only	8	3	5	1	5	9	31
IgG only	7	0	12	6	16	73	114
IgA and IgG	48	10	6	4	19	16	103
None	15	1	9	11	70	392	498
Total	78	14	32	22	110	490	746

TABLE II *Results of immunofluorescent-antibody test to detect antigonococcal IgA in cervical secretions from 78 women with untreated gonorrhoea compared with those of Gram-stain microscopy and culture*

Fluorescent-antibody test	*Positive results to direct microscopy and culture*			*Total*
	Gram-stain only	*Culture only*	*Gram-stain and culture*	
Antigonococcal IgA detected	1	16	39	56
Antigonococcal IgA not detected	0	4	18	22
Total	1	20	57	78

IgG

IgG antibody reactive with *N gonorrhoeae* was demonstrated in the cervical secretions of 55 (71%) of 78 women with untreated gonorrhoea and in 89 (18%) of 490 non-infected patients. This antibody was found in the secretions of 19 (17%) of the 110 women who had been treated for gonorrhoea at least 29 days previously.

COMPARISON WITH GRAM-STAIN MICROSCOPY

In relation to the results of culture, the sensitivity (72%) of the fluorescent-antibody test with antiserum against IgA was almost identical to that of Gram-stain microscopy (74%) (table II).

ORAL CONTRACEPTIVES AND THE FLUORESCENT-ANTIBODY TEST

Data on the method of contraception, if any, were available for 60 women with gonorrhoea. Table III shows the results of the fluorescent-antibody test with IgA antiserum in relation to the contraceptive method used. Antigonococcal IgA was detected in 22 (73%) of 30 women who used an oral contraceptive preparation but in only 12 (40%) of 30 patients who used some other form of contraception or none. This difference in antibody detection is statistically significant ($\chi_1^2 = 5\cdot50$; $P<0\cdot02$).

TABLE III *Influence of methods of contraception on the detection of antigonococcal IgA in cervical secretions from women with gonorrhoea*

	Results of fluorescent-antibody test		
Type of contraception	*IgA present*	*IgA absent*	*Total*
Oral	22	8	30
IUD or none	12	18	30
Total	34	26	60

IUD = intrauterine device

Discussion

In a previous study,[7] IgA reactive with *N gonorrhoeae* was demonstrated in the cervical secretions (obtained by aspiration) of 71 (95%) of 75 women with untreated gonorrhoea. The rate of detection of antibody (72%) was significantly lower in the present series and probably reflects the different sampling method; the mean IgA concentration in the secretions eluted from the swabs was $11\cdot4\times10^{-3}$ g/l compared with $24\cdot0\times10^{-3}$ g/l in secretions obtained by aspiration.[7] The collection of secretions by the latter method is however cumbersome and not suitable for routine use in a busy clinic.

The sensitivities of the immunofluorescent-antibody test with IgA antiserum and of Gram-stain microscopy were almost identical (72 and 74% respectively) but the former test was expensive and time-consuming, and results were not available for at least one hour after collection of specimens. Similar difficulties were encountered with direct fluorescent-antibody procedures.[11]

Although antigonococcal IgA was detected in secretions from 25 (5%) of 490 women who had no microbiological evidence of gonococcal infection, it cannot be assumed that these patients had not recently been infected. In previous studies,[7,12] IgA antibody was not detectable in the cervical secretions of women who had been treated more than 14 days previously. From the present study however, it is clear that this antibody can be detected in the secretions a month or even longer after satisfactory treatment. It is possible that some of these 25 women had been treated inadvertently before attending the clinic. As previously shown,[7] a local antibody response may be detected in known contacts in whom repeated microbiological examination fails to show active gonococcal infection.

It was of interest that antigonococcal IgA was detected significantly more frequently in cervical fluid from women with gonorrhoea who used an oral contraceptive preparation. Chipperfield and Evans[13] showed that the mean concentration of IgA in cervical mucus was significantly higher in women using a combined pill than in women with natural cycles.

The low sensitivity and specificity of the fluorescent-antibody test using IgG antiserum is clearly shown. IgG antibody is frequently detected in cervical secretions from patients with nongonococcal cervicitis and is probably the result of transudation of natural serum antibody through the inflamed mucus membrane.

As IgM antibody is detectable in the secretions of only about 40% of women with gonorrhoea,[7] the fluorescent-antibody test with IgM antiserum was not performed.

We wish to thank the staff of the two clinics for their assistance in the collection of specimens.

This project was supported by a grant (reference number K/MRS/50/C182) from the Scottish Home and Health Department.

References

1. Koransky JR, Jacobs NF. Serologic testing for gonorrhea. *Sex Transm Dis* 1977;4:27-31.
2. Kellogg DS, Balows A. Immune response to *Neisseria gonorrhoeae*. In: Rose NR, Friedman H, eds. *Manual of Clinical Immunology*. Washington DC: American Society for Microbiology, 1976:280-4.

3. McMillan A, McNeillage G, Young H, Bain SR. Serum immunoglobulin response in uncomplicated gonorrhoea. *Br J Vener Dis* 1979;**55**:5-9.
4. Wilkinson AE. Indirect fluorescent test for the detection of antigonococcal antibodies. *Br J Vener Dis* 1975;**51**:28-30.
5. Ison CA, Glynn AA. Classes of antibodies in acute gonorrhoea. *Lancet* 1979;i:1165-8.
6. Kearns DH, O'Reilly RJ, Lee L, Welch BG. Secretory IgA antibodies in the urethral exudate of men with uncomplicated urethritis due to *Neisseria gonorrhoeae*. *J Infect Dis* 1973;**127**:99-101.
7. McMillan A, McNeillage, G, Young H, Bain SSR. The secretory antibody response of the cervix uteri to infection with *Neisseria gonorrhoeae*. *Br J Vener Dis* 1979;**55**:265-70.
8. Barlow D, Phillips I. Gonorrhoea in women. Diagnostic, clinical and laboratory aspects. *Lancet* 1978;i:761-4.
9. Samson RR, McClelland DBL, Shearman DJC. Studies on the quantitation of immunoglobulin in human intestinal secretions. *Gut* 1973;**14**:616-26.
10. O'Reilly RJ, Welch BG, Kellogg DS. An indirect fluorescent-antibody technique for study of uncomplicated gonorrhea. II Selection and characterization of the strain of *Neisseria gonorrhoeae* used as antigen. *J Infect Dis* 1973;**127**:77-83.
11. Lind I. Identification of *Neisseria gonorrhoeae* by means of fluorescent antibody technique. *Acta Pathol Microbiol* 1967;**70**:613-29.
12. O'Reilly RJ, Lee L, Welch BG. Secretory IgA antibody responses to *Neisseria gonorrhoeae* in the genital secretions of infected females. *J Infect Dis* 1976;**133**:113-25.
13. Chipperfield EJ, Evans BA. Effect of local infection and oral contraception on immunoglobulin levels in cervical mucus. *Infect Immun* 1975;**11**:215-21.

Br J Vener Dis 1980;**56**:227-9.

One-step staining of Neisseria gonorrhoeae in urethral discharge by methyl green-pyronin

N F HANNA,* D TAYLOR-ROBINSON,* G W CSONKA,† J R W HARRIS,† AND I A AL-SOWAYGH‡

*From the *Division of Communicable Diseases, Clinical Research Centre, Harrow, Middlesex, and the †Praed Street Clinic, St Mary's Hospital, London; and the ‡University of Riyad, Saudi Arabia*

SUMMARY Methyl green-pyronin (MGP) was used in a one-step procedure to stain smears of urethral discharge from 169 men. Duplicate smears were stained by Gram's method and discharge was cultured for *Neisseria gonorrhoeae*. The organisms were isolated from 67 specimens and intracellular diplococci were seen in 74 smears after Gram staining and in 77 after staining by MGP. Furthermore, more extracellular and intracellular diplococci were seen in smears stained by MGP than by Gram's method and the proportion of polymorphonuclear leucocytes found to contain the organisms was greater after staining with MGP. Staining with MGP is simple, rapid, inexpensive, and easily automated.

Introduction

The Gram stain is used routinely to stain urethral smears and requires five steps. It is well known that *Neisseria gonorrhoeae* organisms appear as red diplococci and that the nuclei of polymorphonuclear (PMN) leucocytes are also a shade of this colour. Sowter and McGee[1] were easily able to see gonococci in Fallopian tube tissues by using a Gram-methyl-green-pyronin-light-green (Gram-MGPLG) stain because the organisms were stained scarlet red in contrast to the green-blue staining of the cytoplasm and the blue nucleus of the epithelial cells. We thought, therefore, that this stain, or a slight modification of it (methyl green-pyronin, MGP), might be useful in examining smears from men with urethral discharge. Furthermore, the MGP-staining procedure requires a single step only and it might be useful in private practice and be easily automated for use in busy venereal disease clinics. Accordingly, specimens from men with urethral discharge have been cultured for *N gonorrhoeae* and smears have been stained by Gram's method and by MGP and the results compared.

Patients and methods

URETHRAL SMEARS

One hundred and sixty-nine men presenting with urethral discharge were examined. They were not consecutive cases but were randomly selected on the basis of the presence of a purulent discharge. After a specimen of discharge had been taken from each patient for culture on Thayer-Martin medium, further specimens were collected with a bacteriological loop and spread on two glass slides. Each smear covered a 1-cm^2 area marked on the undersurface of the slide in ink with a rubber stamp. The first smear was stained conventionally with Gram stain and the second smear with MGP stain; for each alternate patient this procedure was reversed—that is, MGP was used first and Gram second.

METHYL GREEN-PYRONIN (MGP) STAIN

The MGP stain was prepared by dissolving 0·15 g of methyl green (CI 42585) and 0·5 g of pyronin (CI 45005) in 2·5 ml of 96% ethyl alcohol; 20 ml of glycerin and 100 ml of a solution of crystalline phenol (Analar) (0·5% w/v in distilled water) were then added. The solution was mixed with 100 ml of chloroform and allowed to stand at room temperature overnight. The mixture separated into two phases and the top layer, which comprised the

Address for reprints: Dr D Taylor-Robinson, Division of Communicable Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex

Received for publication 21 November 1979

stain, was removed carefully to avoid contamination with the chloroform. The stain was returned to the bottle at the end of a working day and shaken. Each batch of stain was used for a period of six months. All dyes were obtained from Raymond A Lamb Ltd (6 Sunbeam Road, London NW10 6IL).

FIXATION AND STAINING PROCEDURES

Smears of urethral discharge on glass slides were fixed by heat before being Gram stained. Smears for MGP-staining were dried in air; these slides were then immersed for two minutes in MGP stain contained in a coplin jar, rinsed in tap water, and dried.

READING OF SMEARS

MGP-stained and Gram-stained smears were read by two experienced observers, who each used a separate microscope at × 1000 magnification. One hundred smears were examined by one observer, 69 by the other, and a few smears by both. The reading of smears was limited to the 1-cm^2 area and all intracellular and extracellular organisms within this area were counted, as much time being taken as was required to do this.

Results

OVERALL QUANTITATIVE ASSESSMENT

Urethral specimens from 169 patients were examined. Preliminary assessment indicated that the results obtained by one observer were similar to those obtained by the other; the combined results are therefore presented.

N gonorrhoeae was cultured from specimens taken from 67 patients whose Gram-stained and MGP-stained urethral smears also contained intracellular pink diplococci (table). Of the 102 culture-negative specimens, seven had given a positive result by Gram stain and 10 a positive result by the MGP stain. Only four of these specimens gave positive results by both staining techniques. All specimens which were culture-positive showed diplococci by one of the two staining methods.

TABLE *Isolation of N gonorrhoeae from the urethral discharge of 169 men and the occurrence of Gram-stained or MGP-stained intracellular and extracellular diplococci in their urethral smears*

	No of urethral specimens	
Positive results to staining by	*Culture +*	*Culture −*
Gram's method (negatively stained)		
Intracellular diplococci	67	7
Extracellular diplococci	67	2
MGP		
Intracellular diplococci	67	10
Extracellular diplococci	67	6
Both methods	67	4
Total	67	102

+ Positive − negative

QUALITATIVE ASSESSMENT

PMN leucocytes containing diplococci

The proportion of PMN leucocytes seen to contain pink diplococci was greater after the smears were MGP-stained than after they were Gram-stained (fig 1). In addition, the number of PMN leucocytes containing >10 diplococci was greater when the smears were MGP-stained than when they were Gram-stained (fig 2). Indeed, the distribution of results was very similar to that shown in fig 1.

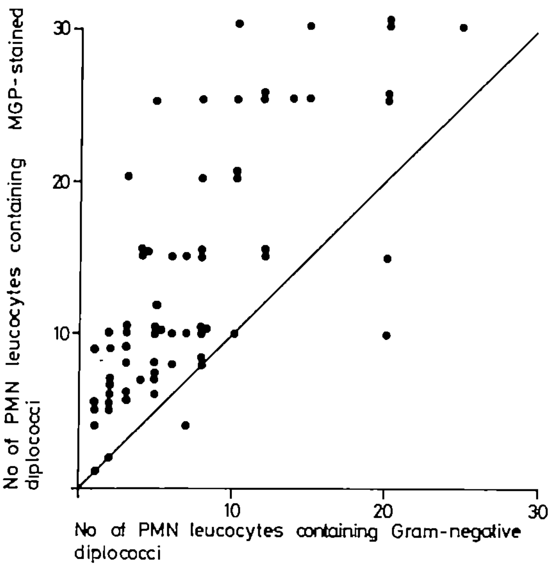


FIG 1 *Urethral smears from 67 N gonorrhoeae-culture-positive patients. The number (mean) of PMN leucocytes per high-power field (× 1000) of each smear containing diplococci that stained Gram-negatively are compared with the number containing diplococci that stained with MGP.*

Extracellular diplococci

Extracellular, as well as intracellular, diplococci were seen in the urethral smears of all patients whose discharge was culture-positive, whether the smears were Gram-stained or MGP-stained (table). However, more extracellular diplococci were seen in MGP-stained smears than in Gram-stained smears (fig 2). In addition, a few of the culture-negative specimens containing intracellular diplococci also had extracellular organisms which stained by one of

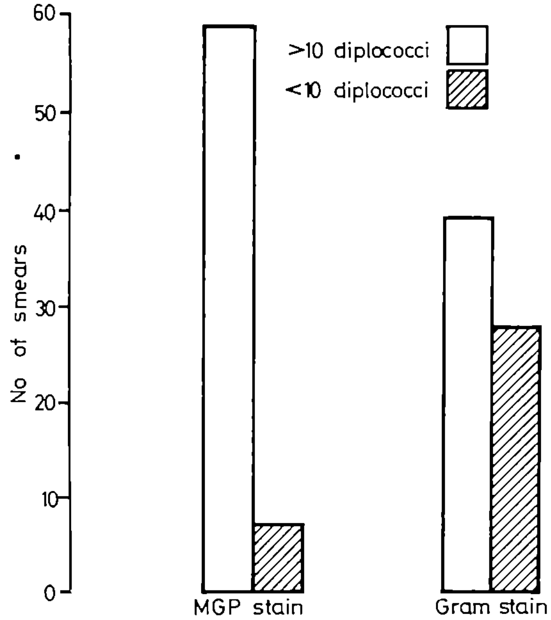


FIG 2 *Urethral smears from 67 N gonorrhoeae-culture-positive patients. Number of smears containing >10 and <10 extracellular diplococci after staining with MGP and Gram's method.*

the two methods (table). Five culture-negative specimens containing Gram-negative extracellular organisms only and four containing MGP-stained extracellular organisms only are not shown.

Discussion

Methyl green-pyronin stains gonococci red and the nuclei of the cells blue. It has, therefore, a greater differentiating capacity than methylene blue, which does not stain organisms or cell components differently. Furthermore, it is easier to detect gonococci within a cell using the MGP stain than it is using the Gram stain where organisms, particularly those overlying the nucleus, may not be seen. This may be the reason why more diplococci were seen within cells after staining with MGP. It is, however, difficult to explain why larger numbers of extracellular diplococci were seen after MGP-staining than after Gram-staining. It is possible only to speculate that organisms take up the MGP stain more avidly, and are therefore more easily seen, or that inadequate decolorisation in the Gram-stain procedure results in some organisms appearing Gram-positive.

We have found that methanol or formol-saline are superior to other fixatives because they increase the clarity of cell components. However, in this study, heat and air fixation provided sufficient clarity and were simple to perform.

The only problem using the MGP stain, as with methylene blue, is that it differentiates poorly between Gram-positive and Gram-negative organisms. Although organisms which are strongly Gram-positive may appear magenta colour with the MGP stain, while Gram-negative organisms appear red, the unwary observer may occasionally mistake a Gram-positive organism for a gonococcus. This may account for some of the "false-positive" results recorded for a few of the specimens in our series—that is, those where MGP stained intracellular organisms in a smear but gonococci were not isolated in culture. However, the number of such false-positive results produced by MGP-staining was only three more than by Gram-staining. It may be that these were due to the presence of commensal neisseriae or that the so-called false-positive results were not truly false but due to a relative failure to culture the organisms. A better assessment of the rate of false-positive results might be made by comparing the results of staining smears with Gram's method or MGP with those of staining duplicate smears with gonococcal fluorescent antibody, although differentiation between *N gonorrhoeae* and *N meningitidis* would still be difficult. Overall, we regard the problem of false-positive results with the MGP stain as a minor one, and it does not in any way outweigh the value of this staining procedure, which is both rapid and simple. In addition, the MGP-staining procedure is less costly than Gram staining because the components are cheaper and can be reused for a period of up to six months. MGP staining is also more easily automated since it involves only a single step.

We thank Mr C Sowter (Histopathology Department, Clinical Research Centre, Harrow) for advice on preparation and use of the MGP stain; the medical attendants and nursing staff of the Praed Street Clinic for their help; and the staff of the Department of Bacteriology, St Mary's Hospital Medical School, London, for culture facilities.

Reference

1. Sowter C, McGee ZA. Evaluation of a new technique for the demonstration of gonococci and other organisms in host cells. *J Clin Path* 1976;29:433-7.

Br J Vener Dis 1980; **56**: 230-4.

Induction of a cell wall variant of the gonococcus by human amylase

A MELLERSH, S HAFIZ, M G McENTEGART, AND I GEARY

From the Department of Medical Microbiology, University of Sheffield Medical School, Sheffield

SUMMARY Human amylase has been shown to be an effective and powerful inhibitor of the gonococcus in vitro. Its action appears to be on the bacterial cell wall. When tested in osmotically stabilised liquid media the organism was not inhibited; however the organisms which grew in such cultures appeared to be cell wall variants, which were less sensitive to penicillin than the parent strains. Studies on these variants suggest that they are "transitional-phase variants." Since cervical mucus has much in common with an alkaline osmotically stabilised liquid medium and also contains a high concentration of amylase, it seems possible that these variants may occur in vivo.

Introduction

Normal human saliva is a potent and specific inhibitor of the gonococcus in vitro, and this effect has been shown to be due to amylase.[1] High concentrations of amylase are found in saliva, cervical mucus,[2] and seminal fluid,[3] and yet the gonococcus appears to survive in areas bathed by these fluids.

Because of its high molecular weight, about 50 000 daltons, it seems probable that amylase acts at the level of the outer cell wall and does not penetrate the bacterium. Inhibitors of bacteria that act at the level of the cell wall are known to induce cell-wall-defective, osmotically fragile variants.[4]

A major difference between growth of the gonococcus in vivo and in vitro is that in the former the body supplies a degree of osmotic stability; we felt that this protection—of an osmotically fragile, cell-wall-defective variant—might explain the survival of the gonococcus in the areas containing high concentrations of amylase.

Materials and methods

MEDIA

The liquid medium used was ANM,[5] and this was osmotically stabilised by the addition of 1% polyvinylpyrrolidone (BDH, Analar; molecular weight, 700 000); this is referred to as the liquid medium. Subcultures were made on to plates of Difco GC agar base with 2% defined supplement. When plates were to be osmotically stabilised 1% polyvinylpyrrolidone (BDH) was added. Subcultures from the liquid medium were made on both osmotically stabilised (OS) plates and non-stabilised (NOS) plates. Normally, ANM has a pH of 7·2, but some of the liquid media was adjusted with sodium hydroxide, (1 mol/l) to a pH of 8·0 (the pH of the cervical mucus of a woman on the contraceptive pill).

N GONORRHOEAE STRAINS

International strain WHO VII, β-lactamase producers HKI and RI, as well as recent isolates from the Sheffield Special Clinic, were used for this study.

AMYLASE EXTRACTION

Amylase was extracted from pooled human saliva by the method of Meyer *et al.*[6] Purified amylase was added to the liquid media to give a final amylase activity of approximately 2000 Somogyi units per ml.

STAINING

Cultures were centrifuged at 2000 rev/min for 10 minutes and the deposit washed with phosphate-buffered saline, placed on slides, and stained using Dienes or Gram stain.

MILLIPORE FILTRATION

Eighteen-hour cultures in stabilised liquid medium were passed through 0·45μ Millipore filters and the filtrate cultured in stabilised liquid medium as well as on OS and NOS plates.

Address for reprints: Professor M G McEntegart, Department of Medical Microbiology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX

Received for publication 14 May 1979

IMMUNOFLUORESCENCE

A loopful of suspension prepared from the centrifuged deposit was allowed to dry in air on a standard microscope slide, fixed in acetone, and covered with a 1/16 dilution of antigonococcal immunofluorescent conjugate (Bacto-FA *N gonorrhoeae* Difco). Slides were left in a moist box for 15 minutes, washed in distilled water, dried, and mounted in glycerol buffer for examination.

ELECTRON MICROSCOPY

Centrifuged deposits were suspended in 3% buffered gluteraldehyde for one hour and then post-fixed with 1% osmium tetroxide for 30 minutes, prestained with uranyl acetate for 30 minutes, and dehydrated through increasing concentrations of ethyl alcohol to epoxy propane and finally embedded in epoxy resin. Ultra-thin sections were cut using a Reichert OMU 3 ultramicrotome. The thickness of sections ranged from 60 to 80 nm. The sections were mounted on copper grids and stained with Reynolds lead citrate.

ENDOTOXIN ASSAY (CHICK EMBRYO TESTS)

Eighteen-hour cultures of WHO VII strain gonococci grown in the liquid medium with amylase were centrifuged and the deposit resuspended in saline and standardised with an EEL nephelometer to contain 10^6 organisms per ml. For the test, 10-day-old embryonated eggs were used. These were kept in a humidified incubator at 37°C. For inoculation, a rectangular window (approximately 3 × 5 mm) was cut over a prominent allantoic vein (while candling) and the shell flap was lifted off with a needle. After the windows had been cut the eggs were undisturbed for one hour to minimise bleeding on inoculation; 0·1 ml of the suspensions was inoculated into each egg with a 27-gauge needle. Control eggs received a suspension of vegetative-phase bacteria at the same concentration or, as a negative control, phosphate-buffered saline. After inoculation, the eggs were returned to the incubator for two days and were candled at 24 and 48 hours. When death of the embryo was suspected, it was confirmed by immediate examination of the contents of the egg. Specific endotoxin deaths showed very characteristic pathological changes and usually occurred in the first 24 hours after inoculation.

PENICILLIN SENSITIVITY

Benzyl penicillin was diluted in the liquid medium to give a series of dilutions from 1·0 to 0·0025 units per ml as shown in the table. Amylase and 0·1 ml of an 18-hour culture of WHO VII strain gonococci (in the liquid medium with amylase) were added to each dilution. WHO VII strain gonococci grown without amylase and the Oxford Staphylococcus were added to two similar series of penicillin dilutions as controls. Penicillinase production was detected by the addition of a chromogenic cephalosporin (Nitrocefin, Glaxo).

TABLE *Sensitivity of two strains of N gonorrhoeae and the Oxford Staphylococcus to benzyl penicillin*

	Concentrations of penicillin (units per ml)					
Organisms	*1·0*	*0·1*	*0·05*	*0·01*	*0·005*	*0·0025*
Staphylococcus Oxford (control)	–	+	+	+	+	+
WHO VII vegetative phase (non-amylase treated)	–	–	–	–	+	+
WHO VII variant phase (amylase-treated)	–	+	+	+	+	+

+ Growth – no growth

Results

CULTURE GROWTH AND STAINING

In the osmotically stabilised liquid media, all strains showed visible growth in 18 hours. Subcultures made on OS and NOS plates at 18 hours all grew. Colonies were more numerous on OS plates, especially on subcultures made more than 48 hours after inoculation of the liquid media. On some occasions no growth was obtained on NOS plates after 48 hours. When examined in wet preparation the growth in the liquid medium was typically seen as clumps of organisms up to 2 mm in diameter. Growth in the medium at pH 8·0 was similar to that at 7·2. The colonial forms on primary subculture from the liquid medium on to solid medium were of three types. Most were typical gonococcal colonies. A varying number, up to 50%, were atypical with a raised circular centre and a flat periphery with a well defined border. Subsequent subcultures from these colonies yielded typical gonococcal colonies of Gram-negative diplococci which fermented glucose but not maltose or sucrose. About 1% of colonies were of the L form. Dienes-stained films of 18-hour cultures showed aggregates of weakly staining granular material with a few darker staining diplococci (fig 1). These aggregates were Gram-negative and, when tested with a known antigonococcal serum, showed bright on specific fluorescence (fig 2). Millipore filtration was unsuccessful in that only a few viable units passed through the filter.

ELECTRON MICROSCOPY

On electron microscopy the control organisms incubated without amylase (fig 3) had a uniform shape, typical cell wall structure, and an osmophilic cytoplasm indicating a large number of ribosomes.

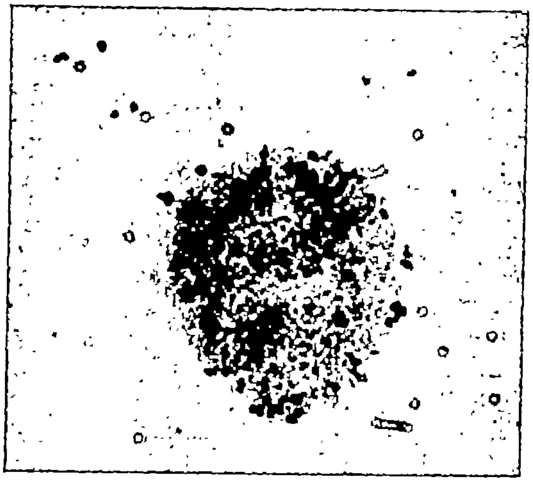


FIG 1 *Impression preparation of an L-form colony grown from a subculture of gonococci exposed to amylase in liquid medium for 18 hours. Dienes stain ×1230.*

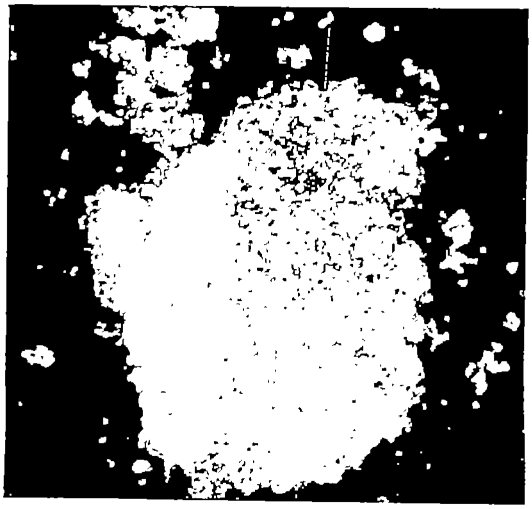


FIG 2 *Impression preparation of L-form colony showing bright fluorescence when stained with a specific antigonococcal conjugate. ×1230.*

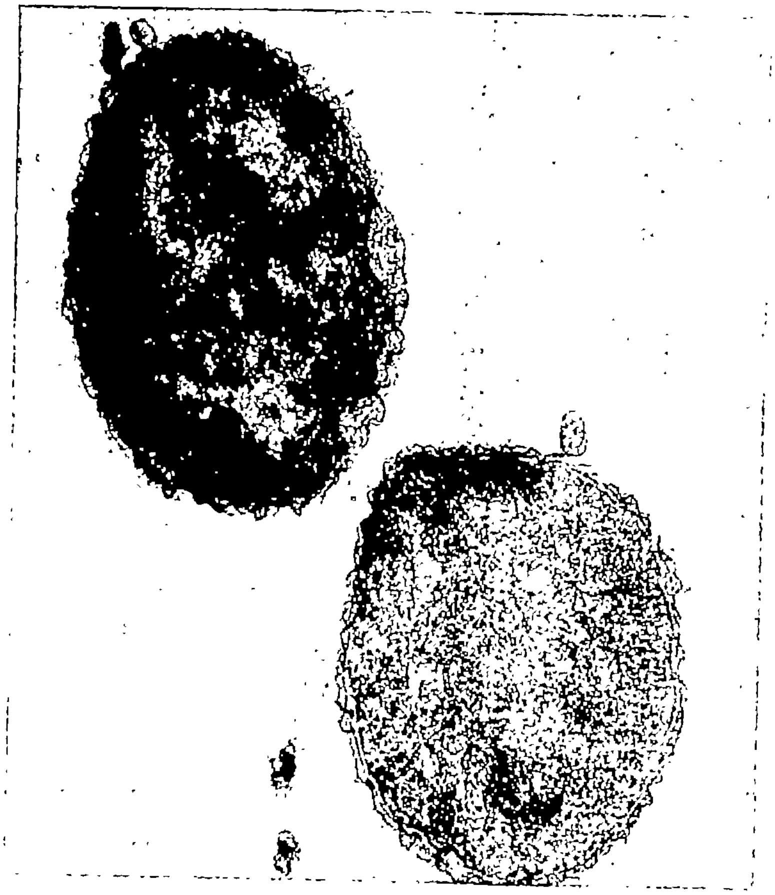


FIG 3 *Electron micrograph (×54 500) showing normal appearance of control gonococcus.*

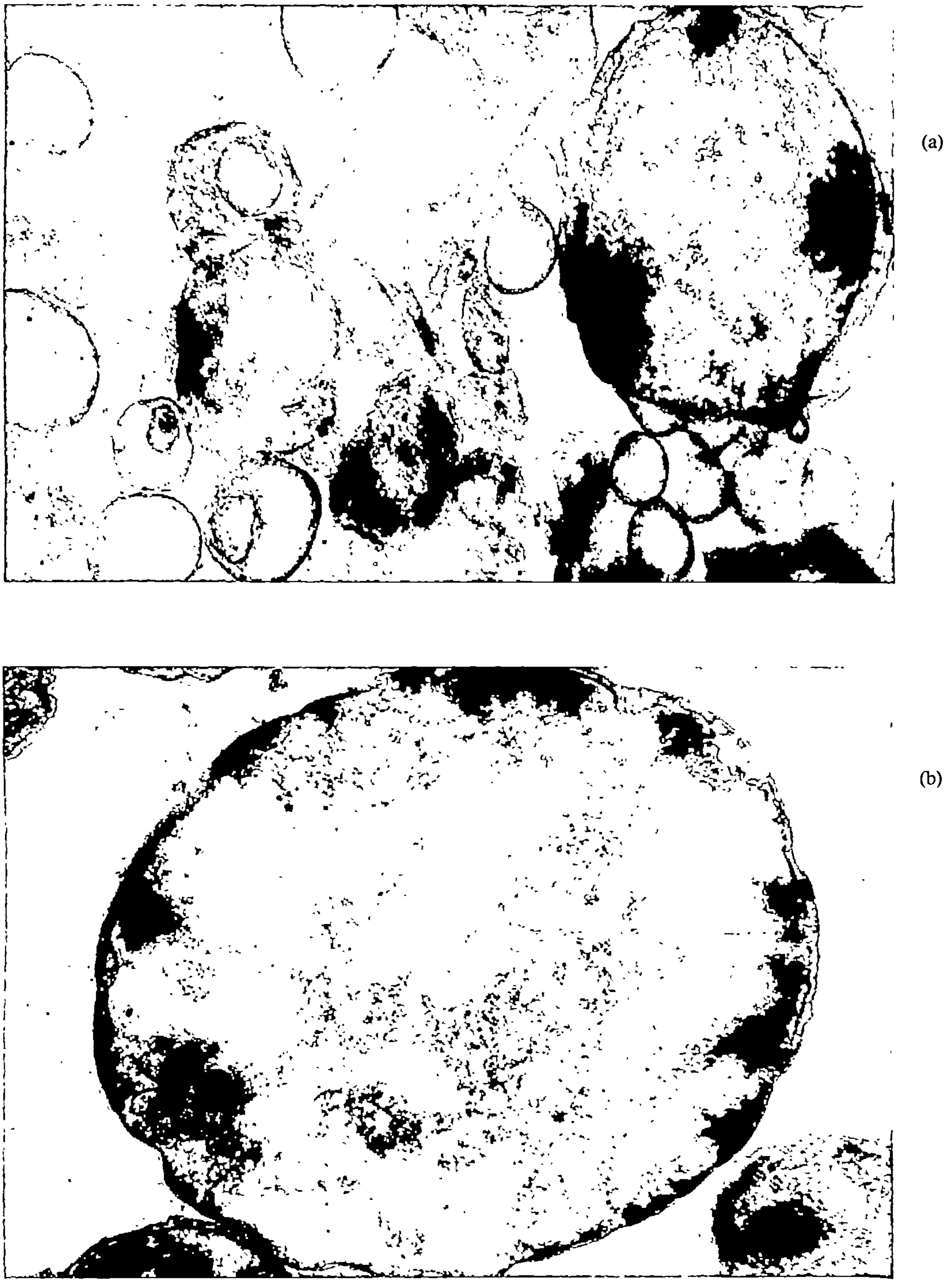


FIG 4 (a) and (b) *Electron micrographs (×54 500) of amylase-treated gonococci showing gross variation in cell size and damage to the cell wall.*

They showed typical endotoxin blebs. Organisms incubated with amylase were irregular in shape and size (fig 4); they were limited by a simple cell wall, often just a cytoplasmic membrane. This membrane was often duplicated and the inner membrane discontinuous. In larger organisms the osmophilic material was less and scattered at the periphery. Numerous smaller units resembling the elementary bodies of L-phase variants were observed, but these were presumably not viable, as viable units were rarely obtained after Millipore filtration. No endotoxin blebs were observed.

ENDOTOXIN ASSAY

The organisms grown in the liquid medium containing amylase were toxic to chick embryos (70% endotoxic deaths) but not as toxic as the vegetative-phase bacteria (90% endotoxic deaths). No deaths were observed in the controls. (Each of the three groups consisted of 30 chick embryos.) The growth in the liquid media with amylase was still sensitive to penicillin but not as sensitive as the vegetative-phase bacteria (table). β-lactamase-producing bacteria continued to produce β-lactamase throughout the experiments.

Discussion

Although the variant induced by amylase in these experiments resembles an L-phase variant on electron microscopy, there are some important differences. This variant is fast growing whereas the L-phase variant grows slowly. This variant is sensitive to the action of penicillin whereas the L-phase variant is, by definition, resistant. This variant cannot reproduce serially in a distinctive colonial form on agar. We feel, therefore, that it satisfies the criteria of a "transitional variant" as defined by McGee *et al.*[7] Ota *et al*[8] studied reverting L-phase variants on agar and observed an intermediate colonial form which was strikingly similar to those forms we observed on subculture of these variants. However, these workers[8] did not attempt to grow it in liquid media, study its properties, or derive it directly from vegetative bacteria.

Osmotically fragile cell wall variants have been shown to occur in clinical gonorrhoea[9] and organisms resembling L-phase variants on electron microscopy have been observed in clinical specimens.[10] These variants have been assumed to be L-phase variants, although this is inconsistent with the clinical fact that penicillin cures gonorrhoea. If the osmotically fragile fraction was transitional in nature, this would be more consistent, since this transitional variant is sensitive to penicillin.

The cervical mucus is osmotically stabilised, contains amylase in high concentration, and is alkaline. Attempting to simulate these conditions in vitro, we obtained a transitional-phase variant, and we consider therefore that it is likely that these variants occur in vivo and may have a part in explaining the relatively asymptomatic nature of infections of the uterine cervix.

References

1. Mellersh A, Clark A, Hafiz S. The inhibition of *N gonorrhoeae* by normal human saliva. *Br J Vener Dis* 1979;**55**:20-3.
2. Gregoire AT, Rankin J, Rakoff AE, Adams A. α-amylase content in cervical mucus of females receiving sequential, non-sequential, and no contraceptive therapy. *Fertil Steril* 1967;**18**:836-9.
3. Goldblatt MW. Constituents of human seminal plasma. *Biochem* 1935;**29**:1346-57.
4. Butler HM, Blakey JL. A review of bacteria in L-phase and their possible clinical significance. *Med J Aust* 1975;**2**:463-7.
5. Hafiz S, McEntegart MG. Prolonged survival of *N gonorrhoeae* in a new liquid medium. *Br J Vener Dis* 1976;**52**:381-3.
6. Meyer KH, Fischer H, Bernfeld P, Staub A. Purification et crystallisation de l' α-amylase de salive. *Experientia* 1947;**3**:455.
7. McGee ZA, Whittler RG, Gooder H, Charache P. Wall defective microbial variants: terminology and experimental design. *J Infect Dis* 1971;**123**:433-8.
8. Ota F, Fukui K, Ashton FE, Diena BB. The fine structure of reverting L-forms of *Neisseria gonorrhoeae*. *Jpn J Microbiol* 1976;**20**:59-62.
9. Gnarpe H, Wallin J. Studies in venereal disease. II Improved diagnosis of gonorrhoea by parallel use of conventional and L-phase media for culture. *Br J Vener Dis* 1973;**49**:505-7.
10. Ovcinnikov, NM, Delektorskij VV. Electron microscope studies of gonococci in the urethral secretions of patients with gonorrhoea. *Br J Vener Dis* 1971;**47**:419-39.

Br J Vener Dis 1980; **56**: 235-8.

Bacterial interference of Neisseria gonorrhoeae by α-haemolytic streptococci

M E McBRIDE, W CHRISTOPHER DUNCAN, AND JOHN M KNOX

From the Department of Dermatology, Baylor College of Medicine, Houston, Texas, USA

SUMMARY Fifty pharyngeal isolates of α-haemolytic streptococci were tested against 20 cervical isolates of *Neisseria gonorrhoeae* for bacterial interference in vitro using the lawn-spotting method. Forty-seven (94%) isolates of streptococci showed inhibitory activity toward *N gonorrhoeae,* although nine of these were inhibitory to only one isolate of *N gonorrhoeae.* Isolates of *N gonorrhoeae* varied widely in their sensitivity to streptococci; the most sensitive were inhibited by 40 isolates of streptococci and the least sensitive by only 14 isolates. Species of *Streptococcus* found to inhibit growth of *N gonorrhoeae* were *S mitis, S MG intermedius, S sanguis II, S mutans,* and *S morbillorum.*

Introduction

In a previous study of cervical flora in women with recent sexual contact with men infected with *Neisseria gonorrhoeae,*[1] one woman, who had a negative culture result for *N gonorrhoeae,* was found to have a high population of α-haemolytic streptococci in the cervix. This isolate of α-haemolytic streptococci inhibited *N gonorrhoeae* in vitro. Since α-haemolytic streptococci are known to inhibit a variety of micro-organisms,[2] including *Neisseria* species,[3] we decided to survey a larger number of strains of each of these organisms for evidence of bacterial interference and of their prevalence and to establish whether or not there were differences in susceptibility between various isolates of *N gonorrhoeae.*

Materials and methods

BACTERIAL STRAINS

Fifty isolates of α-haemolytic streptococci were obtained from pharyngeal swabs from healthy men and women between the ages of 20 and 50 by culture on Casman's sheep blood agar. Single-colony isolates were selected on the basis of their α-haemolytic activity; frequently more than one strain, as differentiated by colonial morphology, was isolated per person. Isolates were identified by the following tests using the scheme of Facklam[4]: optochin sensitivity and bile solubility, bile esculin hydrolysis, growth in 6·5% NaCl, growth in 5% sucrose agar and broth, hippurate hydrolysis, and use of mannitol, lactose, inulin, esculin, and raffinose. Cultures were frozen at −4°C in trypticase soy broth (Difco) containing 20% glycerol and also maintained on Casman's sheep blood agar (Baltimore Biological Laboratories, Maryland, USA) at 4°C.

Isolates of *N gonorrhoeae* were obtained from the Houston Public Health Laboratory from cervical cultures of patients reporting with suspected gonococcal infections. Cervical swabs were immediately plated on to Thayer-Martin medium and incubated in CO_2 for 18-24 hours. Oxidase-positive colonies of Gram-negative diplococci were presumptively identified as *N gonorrhoeae,* subcultured on to chocolate agar, and incubated at 37°C in CO_2 jars. After 18-24 hours' incubation, growth was used immediately for bacterial interference experiments (first subculture). This growth was also suspended in trypticase soy broth (BBL) containing 20% glycerol and stored at −70°C. Chocolate agar was prepared by adding 5% sheep blood to BBL blood agar base and heating in a boiling waterbath for 10 minutes.

BACTERIAL INTERFERENCE

Uniform suspensions were made from the first subculture of *N gonorrhoeae* in phosphate-buffered saline, pH 7·2, measuring turbidity visually against McFarlane tube 2. Lawns were prepared by swabbing the suspension on to the surface of chocolate agar

Address for reprints: Dr M E McBride, Department of Dermatology, Baylor College of Medicine, 1200 Moursund, Houston, Texas, USA

Received for publication 4 June 1979

plates. Isolates of α-haemolytic streptococci were incubated overnight on Casman's blood agar and spotted directly on to the surface of the lawns; the plates were then incubated in CO_2 jars at 37°C for 18-24 hours. Zones of inhibition of growth of *N gonorrhoeae* were measured from the edge of the streptococcal growth for the purpose of checking the consistency of the observations in repeated trials.

Results

Fig 1 shows zones of inhibition of growth of *N gonorrhoeae* around inoculation of α-haemolytic streptococci, demonstrating varying degrees of inhibition; zones varied between 1 and 5 mm. The inoculum was not quantitated, but certain isolates of α-haemolytic streptococci were found repeatedly to produce larger zones of inhibition than others.

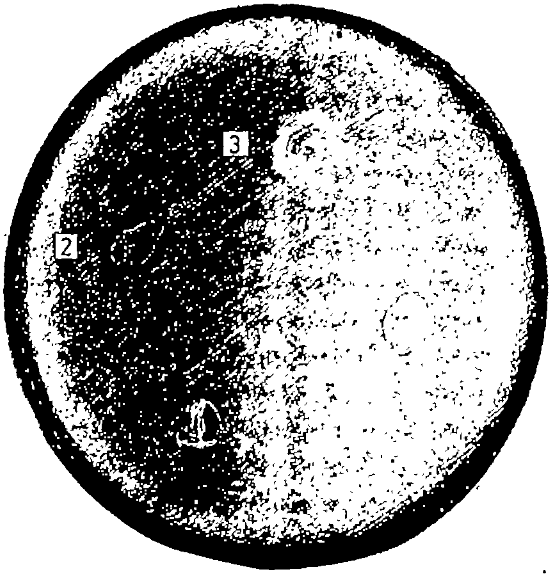


FIG 1 *Four different isolates of α-haemolytic streptococci spotted on lawns of N gonorrhoeae showing varying degrees of inhibition of growth.*

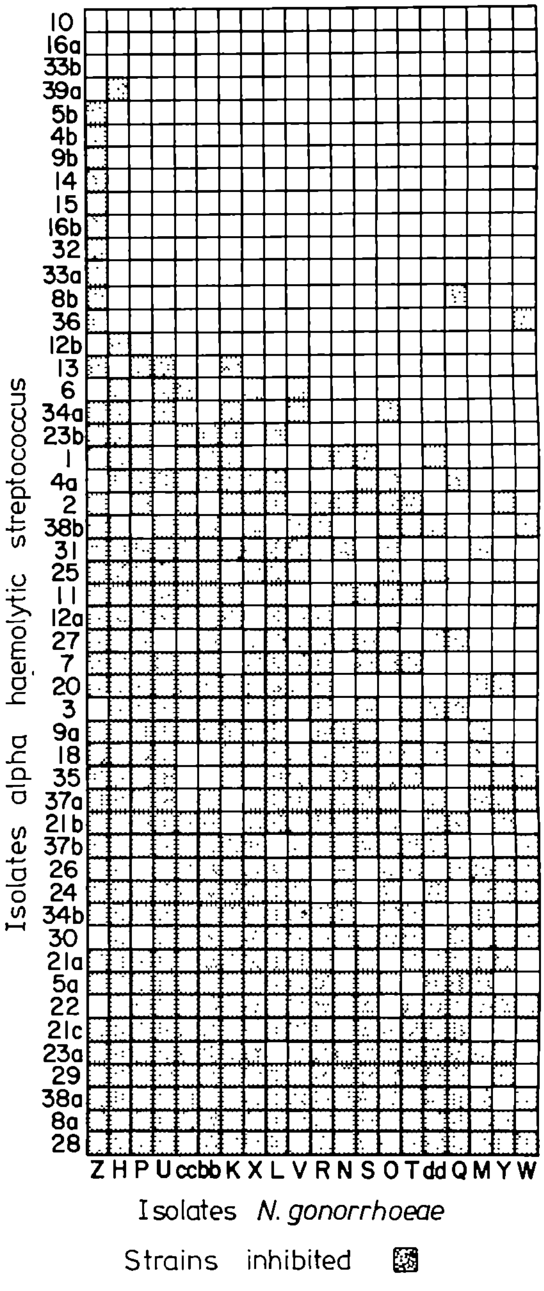


FIG 2 *Inhibition pattern of 20 isolates of N gonorrhoeae by 50 isolates of α-haemolytic streptococci.*

Not all isolates of α-haemolytic streptococci inhibited *N gonorrhoeae;* similarly, different isolates of *N gonorrhoeae* varied in their sensitivity to α-haemolytic streptococci. This is shown in fig 2, in which 50 isolates of streptococci have been arranged along the ordinate in order of their decreasing inhibitory activity, measured by the number of strains of *N gonorrhoeae* they inhibit. Isolates of *N gonorrhoeae* are arranged along the abscissa in order of their decreasing sensitivity to α-haemolytic streptococci. Only three of the 50 isolates of α-haemolytic streptococci were without inhibitory activity to any of the 20 isolates of *N gonorrhoeae* tested; a further nine inhibited only one isolate of *N gonorrhoeae* (fig 2). On the other hand, 18 (36%)

isolates of α-haemolytic streptococci inhibited at least 16 (80%) isolates of *N gonorrhoeae*, even though only one isolate of streptococci inhibited all 20 isolates of *N gonorrhoeae*.

Isolates of *N gonorrhoeae* varied greatly in their sensitivity to the inhibitory activity of α-haemolytic streptococci; the strain designated Z was inhibited by 40 of the isolates of streptococci whereas strain W was inhibited by only 14 (fig 2). It was interesting that some of the isolates of streptococci which inhibited the largest number of isolates of *N gonorrhoeae* (that is, 22) did not inhibit the growth of the most sensitive strain (Z).

The distribution of species of the pharyngeal isolates of α-haemolytic streptococci are shown in table I. *S mitis* was the predominant species, followed by *S sanguis* II and *S MG intermedius*. Inhibitory activity of streptococcal isolates toward *N gonorrhoeae* was not associated with any single species; both active and inactive strains of all species listed in table I were found. Table II shows the frequency of isolation of different strains within species of streptococci showing inhibition toward *N gonorrhoeae*. Of *S mitis* strains isolated, 63·6% inhibited more than 50% of strains of *N gonorrhoeae* tested; 50% of *S MG intermedius* strains had similar activity whereas only 22·2% of *S sanguis* II were inhibitory.

TABLE I *Distribution of species of 50 pharyngeal isolates of α-haemolytic streptococcus*

Species	*% Incidence*
Streptococcus mitis	59·10
Streptococcus sanguis II	20·50
Streptococcus MG intermedius	11·40
Streptococcus morbillorum	4·50
Streptococcus mutans	2·00
Streptococcus sanguis I	2·00

TABLE II *Inhibitory activity of different species of streptococci toward N gonorrhoeae*

	% of strains isolated showing	
Species	*Low activity**	*High activity†*
S mitis	36·4	63·6
S MG intermedius	50·0	50·0
S sanguis II	77·8	22·2

*Inhibitory to <50% of strains of *N gonorrhoeae* tested
†Inhibitory to >50% of strains of *N gonorrhoeae* tested

Discussion

Although the α-haemolytic streptococci used in these experiments were a heterogeneous group, it appears that a high percentage of pharyngeal streptococci are capable of inhibiting *N gonorrhoeae*. It is interesting to speculate whether or not this antagonism occurring in vitro could provide protection against infection in vivo. Although inhibition of *N gonorrhoeae* has been demonstrated in vitro by a number of organisms—*Candida albicans*,[5] *Staphylococcus epidermidis*,[6] non-haemolytic streptococcus,[7] and various enteric Gram-negative bacteria[8]—attempts to show that interference provides protection are few. To have a protective effect, α-haemolytic streptococci would need to occur frequently in the normal flora and in large numbers. In a review of seven studies on the normal flora of the vagina and cervix,[9] the isolation rate for *Streptococcus viridans* or α-haemolytic streptococci varied from 0 to 53%. In more recent quantitative studies, α-haemolytic streptococci have still occurred in varying frequencies but in large numbers.[10-12] The true incidence of α-haemolytic streptococci may not yet be accurately recorded, since the use of selective media for vaginal cultures would inhibit its growth; furthermore, an overgrowth of fast-growing bacteria, such as *Proteus* spp or *Escherichia coli*, would mask its presence.

In our previous study,[1] a protective effect by α-haemolytic streptococci against gonococcal colonisation was apparent in only one patient. Recent attempts to relate the presence of certain groups of organisms in the normal cervical flora to protection against *N gonorrhoeae* have shown that *Staph epidermidis* isolates were most active against *N gonorrhoeae* in vitro,[13] although no correlation was looked for between the presence of *Staph epidermidis* and infection with *N gonorrhoeae*. Saigh *et al*[14] attempted to relate the presence of antagonistic organisms, which were mostly non-haemolytic streptococcal species, staphylococcal species, and *Lactobacillus* spp, to infection with *N gonorrhoeae*. The presence of *Lactobacillus* spp was found to correlate with lack of acquisition of *N gonorrhoeae*, which was interpreted as a cause-and-effect relationship. The differences in the isolation rate of *Lactobacillus* spp were also noted in our study of normal cervical flora in relation to gonorrhoea,[1] but this difference was most obvious between the two population groups, public clinic and private patients. Our interpretation was not necessarily one of cause and effect; however, we believed that the lack of *Lactobacillus* spp in the cervical flora of clinic patients infected with *N gonorrhoeae* was either a result of other factors, such as recurrent episodes of gonorrhoea and treatment with antibiotics eliminating *Lactobacillus* spp, or due to the fact that *N gonorrhoeae* itself might have an inhibitory effect on *Lactobacillus* spp—an effect that we were able to demonstrate in one instance.

While α-haemolytic streptococci may not occur in cervical flora sufficiently often or in great enough numbers to provide protection from gonorrhoea, it may play a role in the prevention of gonococcal pharyngitis. Pharyngeal colonisation by *N gonorrhoeae* usually affects less than 10% of populations attending venereal disease clinics.[15] When certain specialised groups are selected, such as homosexual men who claim to have had oral contact with partners infected with *N gonorrhoeae,* the incidence is higher.[16,17]

The authors wish to thank Mrs Ann Gould for the clinical isolates of *N gonorrhoeae* used in this study and Miss Judith Singer for technical assistance.

References

1. McBride ME, Duncan WC, Knox JM. A method for studying the role of indigenous cervical flora in colonisation by *Neisseria gonorrhoeae. Br J Vener Dis* 1978; **54**: 386-93.
2. Dajani AS, Tom MC, Law DJ. Viridins, bacteriocins of alpha-hemolytic streptococci: isolation, characterization, and partial purification. *Antimicrob Agents Chemother* 1976; **9**: 81-8.
3. Dajani AS, Law DJ, Bollinger RO, Ecklund PS. Ultrastructural and biochemical alterations effected by Viridin B, a bacteriocin of alpha-hemolytic streptococci. *Infect Immun* 1976; **14**: 776-82.
4. Facklam RR. Physiological differentiation of viridans streptococci. *J Clin Microbiol* 1977; **5**: 184-201.
5. Hipp SS, Lawton WD, Chen NC, Gaafar HA. Inhibition of *Neisseria gonorrhoeae* by a factor produced by *Candida albicans. Appl Microbiol* 1974; **27**: 192-6.
6. Kraus SJ, Ellison N. Resistance to gonorrhea possibly mediated by bacterial interference. *Appl Microbiol* 1974; **27**: 1014-6.
7. Crowe CC, Sanders E, Longley S. Bacterial interference. II Role of the normal throat flora in the prevention of colonization by group A streptococci. *J Infect Dis* 1973; **182**: 527-32.
8. Kraus SJ, Geller RC, Perkins GH, Rhoden DL. Interference of *Neisseria gonorrhoeae* growth by other bacterial species. *J Clin Microbiol* 1976; **4**: 288-95.
9. Galask RP, Larsen B, Ohm MJ. Vaginal flora and its role in disease entities. *Clin Obstet Gynecol* 1976; **19**: 61-81.
10. Levison ME, Corman LC, Carrington ER, Kaye D. Quantitative microflora of the vagina. *Am J Obstet Gynecol* 1977; **127**: 80-5.
11. Tashjian JH, Coulam CB, Washington JA. Vaginal flora in asymptomatic women. *Mayo Clinic Proc* 1976; **51**: 557-61.
12. Bartlett JG, Onderdonk AB, Drude E *et al.* Quantitative bacteriology of the vaginal flora. *J Infect Dis* 1977; **136**: 271-7.
13. Kaye D, Levison ME. In-vitro inhibition of growth of *Neisseria gonorrhoeae* by genital micro-organisms. *Sex Transm Dis* 1977; **4**: 1-3.
14. Saigh JH, Sanders CC, Sanders WE. Inhibition of *Neisseria gonorrhoeae* by aerobic and facultatively anaerobic components of the endocervical flora: evidence for a protective effect against infection. *Infect Immun* 1978; **19**: 704-10.
15. Noble RC, Cooper RM, Miller BR. Pharyngeal colonisation by *Neisseria gonorrhoeae* and *Neisseria meningitidis* in black and white patients attending a venereal disease clinic. *Br J Vener Dis* 1979; **55**: 14-9.
16. Bro-Jørgensen A, Jensen T. Gonococcal pharyngeal infections. *Br J Vener Dis* 1973; **49**: 491-9.
17. Weisner PJ, Tronca J, Bonin P, Pedersen HB, Holmes KK. Clinical spectrum of pharyngeal gonococcal infection. *N Engl J Med* 1973; **288**: 181-5.

Br J Vener Dis 1980;**56**:239-42.

Epidemiology of gonococcal vulvovaginitis among children in the tropics*

K O ALAUSA AND A O OSOBA

From the Special Treatment Clinic, Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria

SUMMARY In a three-year prospective clinical study, 42 children aged between 1 and 12 years were found to have gonococcal vulvovaginitis. Epidemiological investigations showed that contaminated fomites—particularly bedclothing, underclothing, and towels—and precocious sexual intercourse were the important vehicles of transmission of gonococci to the genital tract of these children.

Introduction

Vulvovaginitis in childhood can be of gonococcal or nongonococcal origin; the latter condition can be caused either by infectious agents, such as *Candida albicans, Trichomonas vaginalis,* diphtheroid bacilli, haemophilus species, streptococcal species, staphylococcal species, *Escherichia coli,* and thread worms, or by non-infectious substances, such as foreign bodies in the vagina and malnutrition in the presence of poor personal hygiene. Gonococcal vulvovaginitis is the most serious of the two conditions and it has been recognised as an important disease in tropical and developing African countries. For example, Lawson and Stewart[1] stated that four out of five cases of vulvovaginitis in the tropics were due to infection with *Neisseria gonorrhoeae,* and Osoba and Alausa[2] found gonococci in 16 of the 17 children with vulvovaginitis in Ibadan. However, vulvovaginitis caused by *N gonorrhoeae* is considered rare in developed temperate countries of the world.[3]

This report summarises our experience of the clinical features and important epidemiological determinants in 42 consecutive cases of gonococcal vulvovaginitis in children seen at the Special Treatment Clinic (STC), University College Hospital, Ibadan, Nigeria, over a three-year period from July 1976 to June 1979.

Patients and methods

STUDY POPULATION

Thirty-eight consecutive prepubertal girls with confirmed gonococcal vulvovaginitis aged between 1 and 12 years, who were referred by other physicians or brought into the STC by their parents or guardian, were included in the study. These children were grouped as the index cases to distinguish them from the four contact cases who were found to have gonococcal vulvovaginitis after being asked by contact-tracers to attend the clinic. Three of the contact cases were sisters of index cases while the fourth was a housemaid in the family of one of the index cases.

CLINICAL INVESTIGATIONS

A detailed history of the onset of symptoms was taken from the patients' escorts. Inquiries about the possibilities of sexual assault, where and with whom the child slept, the bath towel she used, and the type of toilet facilities in the home and school were also made.

The children were physically examined and their nutritional state and standard of personal hygiene were noted. The underwear was closely inspected for blood stains or vaginal discharge. The vulva was examined for the presence of discharge, ulcers, bleeding sites, and evidence of sexual assault; the condition of the hymen was also noted. The vulva was then cleaned with a sterile cottonwool swab and visualised with a nasal speculum for foreign bodies.

LABORATORY INVESTIGATIONS

Two vaginal swabs and one urethral swab were taken and sent immediately to the laboratory for demonstration and identification of the gonococcus,

---

*Presented at the First African Conference on Sexually Transmitted Diseases, Ibadan, Nigeria, 22-24 August 1979

Address for reprints: Dr K O Alausa, Special Treatment Clinic, Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria

Received for publication 23 October 1979

according to the method previously described by Osoba and Alausa.[2] A wet preparation in saline of one of the vaginal swabs was made and examined for yeasts, trichomonads, worms, and ova. Urine specimens were collected for microscopical examination and culture. A specimen of blood was also taken for the Venereal Disease Research Laboratory test.

CONTACT-TRACING

The members of the family of each child were asked to attend the clinic for examination, investigation, and treatment if necessary. The health sister in charge of the clinic was responsible for ensuring that members of the family of infected children attended the clinic. On a few occasions she had to visit the homes of the child's parents before they agreed to attend the clinic. The health sister also gave health education talks to parents, particularly on the modes of transmission and control of childhood gonorrhoea.

Results

CLINIC ATTENDANCES

During the three-year period, a total of 2900 new patients were seen at the STC; these consisted of 1773 male and 1127 female patients. The number of patients who harboured gonococci was 366—251 male patients (14·2% of the 1173 male cases) and 115 female patients (10·2% of the 1127 female cases). Forty-two (36·5%) of the 115 female patients harbouring gonococci were children aged between 1 and 12 years—that is, 11·5% of all cases harbouring gonococci seen at the STC over the three-year period.

AGE DISTRIBUTION

Of the 42 children, 30 (71·4%) were between 1 and 6 years (table I); 25 of these were under school age. All the five children (three index cases and two contact cases) over 10 years had had previous sexual exposure and had a torn hymen on examination.

CLINICAL FEATURES

Vaginal discharge was the single most common symptom that prompted the parents or guardians of 38 of the children to seek medical advice (table II).

TABLE I *Age distribution of 42 children with vulvovaginitis*

	No of patients		
Age group (years)	*Index cases*	*Contact cases*	*Total*
<1	0	0	0
1-3	13	1	14
4-6	16	0	16
7-9	6	1	7
10-12	3	2	5
Total	38	4	42

TABLE II *Clinical features in 42 children with gonorrhoea*

Clinical features	*No of cases*	*% of total cases*
Vaginal discharge	38	90·5
Pruritus vulvae	10	23·8
Dysuria	4	9·5
Fever	3	7·1
Frequency of micturition or enuresis or both	2	4·8
Lower abdominal pain	2	4·8
Vulval ulceration and bleeding	1	2·4
Torn hymen (found on examination)	5	11·9

The duration of the vaginal discharge ranged between two days and three months depending on whether the child was staying with the mother, stepmother, or other relations; usually the mother of the child would seek medical help earlier than would other guardians. In addition, the parents' previous experiences affected their reaction to the child's symptoms before seeking medical advice; generally, a mother with previous experience or knowledge of the disease would bring her child to hospital early. The only child seen during examination with vulval ulceration and bleeding was staying with her father, who was applying an antiseptic solution to cleanse the child's vaginal discharge; he decided to seek medical help after two weeks when there was no improvement in the child's condition.

FAMILY CONTACTS

In each of the two families where three cases of prepubertal gonorrhoea were found per family (table III), there was an older child of between 10 and 15 years with previous experience of sexual intercourse and gonorrhoea; one of these older girls was a housemaid aged 15 years (who was not included in the study).

SOURCE OF INFECTION

The results of family investigations to determine the probable source of infection among the 38 index cases are shown in tables IV and V. Only 22 mothers and 17 fathers of the 34 families involved responded to our request to attend the clinic.

The role played by older women in the transmission of gonorrhoea within the family is well illustrated. Mothers, sisters, and housemaids

TABLE III *Family cluster of children with prepubertal gonorrhoea*

Cases per family	*No of families*	
1	28	(82·4%)
2	4	(11·7%)
3	2	(5·9%)
Total (%)	34	(100%)

TABLE IV *Sources of infection among the 38 index cases*

Relationship to index case	*No examined*	*No harbouring*		
		Gonococci	*Trichomonads*	*Yeasts*
Mother*	22	8	4	5
Father*	17	4	0	0
Sister	23	3†	1	0
Brother	10	0	0	0
Housemaid	3	2†	1	1
Houseboy	2	0	0	0
Other relatives (stepmother, cousins, aunts)	6	1	0	1
Total	83	18	6	7

*Two couples (mother and father) harboured gonococci
†Three sisters and one housemaid were contact cases

accounted for 13 of the 18 family contacts with gonorrhoea out of the 83 investigated (table V). Although none of the 42 children included in the study had trichomoniasis or candidosis, some of the female members of the families of index cases had these conditions. Four sisters of one index case had scabies but none had gonorrhoea; no other cases of scabies were found.

Discussion

The high rate of positive gonococcal isolation among girls under 12 years attending the STC in Ibadan indicates the importance of gonococcal vulvovaginitis as a public health hazard in Nigeria and other parts of developing tropical Africa. The fact that 36·5% of gonococcal isolates from female patients during the three-year period were from children under 12 years does not however imply that over one-third of female cases of gonorrhoea seen in our hospital are under 12 years. This is because other departments in the hospital continue to see older women with gonorrhoea. Because of the unique position of the STC as the main diagnostic and treatment centre specifically devoted to the management of sexually transmitted diseases in Ibadan, most of the children with vaginal discharge are referred to the STC by physicians all over the city for proper diagnosis and treatment. We are therefore dealing with a highly selective population of children with this infection.

Several factors have been suggested for the apparent high prevalence of childhood gonococcal infection in the tropical developing countries of Africa. Firstly, the ability of the gonococcus to survive longer outside the body in the presence of the high relative humidity (up to 90% during the rainy season) of the tropics, and thus allowing clothes and other fomites contaminated with gonococci to remain infective for at least 3-4 hours, has been demonstrated experimentally by Alausa and others[4] and epidemiologically by Osoba and Alausa[2] in Ibadan. In Nigeria, as in other developing countries, most urban family units consisting of the husband, one or more wives, several children, and other relatives live in crowded one-room or two-room rented shared dwellings in slum areas, and the practice of children and adults sharing the same bedclothing and towels is still very common.

Secondly, in Nigeria, where effective treatment for gonorrhoea is not generally and easily available, the disease is endemic; it is listed as the fifth cause of morbidity when all major notifiable diseases are considered (coming only after malaria, dysentery, measles, and pneumonia).

Thirdly, the socio-economic system in most developing countries still permits child labour, so that older children who cannot be maintained financially by their parents are sometimes hired out as housemaids or houseboys to families in the middle and upper economic strata of the community. These "servants" are usually left at home in charge of children while the parents are at work or attending social functions.

Lastly, polygamy is an acceptable way of living among many of the less educated and poorer people

TABLE V *Probable sources of infection in 42 children with prepubertal gonorrhoea*

Mode/source of infection	*No of cases*	*Clinical and epidemiological findings*
Infected parents (mother or father or both) and relatives (stepmother, cousin, aunt)	12	Sleeping on same bed or mat with parents/relatives and sharing the same bedcover/towel with them. (Parents/relatives were found to have gonorrhoea during family investigation.)
Previous sexual exposure or assault (promiscuity)	5*	Torn hymen discovered at examination. (Evidence of previous sexual intercourse with infected person.)
Infected sisters	4	(Four contact cases.) Elder sisters had gonorrhoea about the same time. Sibs shared underwear with elder sisters.
Infected housemaids	4	A 15-year-old housemaid with gonorrhoea and trichomoniasis was sleeping in same bed with three of the children (aged 2, 4, and 6 years) with gonorrhoea. Another housemaid with gonorrhoea was sleeping in the same bed with another child in the study.
Undetermined source of infection	17	—

*All five cases were aged between 10 and 12 years.

(Yorubas) living in Ibadan. Casual sexual relationships are also common; hence, the risk of disseminating sexually transmitted diseases in the community is very high.

Because of the importance of contaminated fomites—particularly bedclothing, underwear, and towels—and precocious sexual intercourse in transmitting gonococci to the genital tract of Nigerian children, adults who present for treatment of acute gonorrhoea should be warned that they may not only spread infection by sexual contact but may also be a source of danger to young children who may not even come into immediate contact with them.

References

1. Lawson JB, Stewart DB. *Obstetrics and Gynaecology in the Tropics*, 1st ed. London; Edward Arnold Ltd, 1967:411.
2. Osoba AO, Alausa KO. Vulvovaginitis in Nigerian children. *Niger J Paediatr* 1974; 1:26-32.
3. Jeffcoate TNA. *Principles of Gynaecology*, 3rd ed. London: Butterworth Press, 1967:394.
4. Alausa KO, Sogbetun AO, Montefiore D. Effect of drying on *Neisseria gonorrhoeae* in relation to non-venereal infection in children. *Niger J Paediatr* 1977; 4(1):14-8.

Br J Vener Dis 1980;**56**:243.

Penicillinase-producing gonococci in the Netherlands

H BIJKERK

From the Division of Infectious Diseases, Office of the Chief Medical Officer of Public Health, Leidschendam, the Netherlands

Probably most penicillinase-producing strains of *Neisseria gonorrhoeae* (PPNG) isolated in the Netherlands are sent to the Laboratory for Chemotherapy of the Rijks Instituut voor de Volksgezondheid (RIV). The co-operation between the RIV and the Office of the Chief Medical Officer of Health (CMOH) has resulted in a quarterly review of the PPNG strains isolated by the RIV Laboratory for Chemotherapy being sent to the Division of Infectious Diseases of the CMOH. The Division of Infectious Diseases then tries to obtain missing epidemiological data from those who have sent the PPNG strains to the RIV.

The source of infection of the first Dutch case, as described by Stolz,[1] appeared to be a stewardess, who contracted the disease in the Philippines. Since the first two cases in the Netherlands were reported in 1976, the number of cases with infections due to β-lactamase-producing *N gonorrhoeae* increased rapidly (table). In 1979 the number of cases (273) more than quintupled in comparison with that in 1978 (52 cases).

In 1978, PPNG strains were found in four (8%) female patients. The proportion of female cases increased to 25% (67 cases) in 1979.

The proportion of Dutch cases in 1977 was 18% compared with 62% and 45% in 1978 and 1979 respectively.

In the Netherlands, the proportion of these infections contracted outside the country is becoming lower (1976-77, 85%; 1978, 20%; and 1979, 11%) indicating that these strains have become endemic.

Many patients infected in the Netherlands contracted the disease in Amsterdam (1978, 54%; 1979, 58%). The Hague follows with 13% in 1978 and 17% in 1979.

Contact tracing was often unsuccessful; many male patients were infected by prostitutes who were untraceable afterwards.

From the results of a survey carried out by Mr B van Klingeren (Laboratory for Chemotherapy of the RIV), among a number of regional public health laboratories, it is estimated that PPNG strains constitute about 2-3% of all circulating gonococcal strains in the country.

β-lactamase-producing gonococci contain a plasmid which codes for β-lactamase production, such as the "Asian" 4·5 Md and the "African" 3·3 Md plasmids. Van Klingeren *et al*[2] have found that virtually all of the PPNG isolates in 1978 in the Netherlands were found to carry the Asian plasmid. It seems, according to van Klingeren, that this plasmid survives more successfully than the African plasmid.

Address for reprints: Dr H Bijkerk, Division of Infectious Diseases, Office of the Chief Medical Officer of Public Health, Dr Reyersstraat 8, Leidschendam, the Netherlands

Received for publication 26 November 1979

References

1. Stolz E. Penicillinasevormende gonokokkenstam geisoleerd in Rotterdam. *Ned T Geneesk* 1977;**121**:620.
2. Klingeren B van, Embden JDA van, Wijngaarden LJ van. Surveillance of penicillinase-producing gonococci in the Netherlands. *Proceedings of the 11th International Congress of Chemotherapy, 1-5 October 1979, Boston, Mass.*, 1980.

TABLE *Number of infections with β-lactamase-producing N gonorrhoeae by sex (Dutch and foreigners) between 1976 and 1979.*

	Dutch			*Foreigners*			*Total*		
Year	*M*	*F*	*Total*	*M*	*F*	*Total*	*M*	*F*	*Total*
1976	1	1	2				1	1	2
1977	1	1	2	9		9	10	1	11
1978	29	3	32	19	1	20	48	4	52
1979	76	48	124	130	19	149	206	67	273

Br J Vener Dis 1980;**56**:244-8.

Gonorrhoea in Rotterdam caused by penicillinase-producing gonococci

K C NAYYAR,* R C NOBLE,† M F MICHEL,‡ AND E STOLZ*

*From the Departments of *Dermatovenereology and ‡Clinical Microbiology, University Hospital, Rotterdam, the Netherlands; and the †Department of Internal Medicine, University of Kentucky, Lexington, Kentucky, USA*

SUMMARY Recently the prevalence of strains of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) in the Netherlands has increased. A study of demographic and clinical data from patients with gonorrhoea due to PPNG strains, and of the biological characteristics of the strains isolated in Rotterdam, show that Asian strains of PPNG seem at present to be endemic in the Netherlands. Spectinomycin was found to be effective in the treatment of patients with gonorrhoea due to PPNG strains.

Introduction

Penicillinase-producing strains of *Neisseria gonorrhoeae* (PPNG) have been reported from various countries throughout the world.[1] Occasional isolation of these organisms has been reported from several countries in Europe, but more cases have been reported from England and the Netherlands.[1] In England, the outbreak was successfully contained,[2] but in the Netherlands, especially since the beginning of 1979, the number of cases of PPNG infection has increased (Chief Inspector of Public Health, personal communication, 1979). The total number of cases of PPNG infection reported in the Netherlands—a country of 14 million inhabitants—between November 1976 and December 1978 was 65; the figures for the first and second quarters of 1979 were 59 and 60 respectively for cases of PPNG infection and 2390 and 2418 respectively for all reported cases of gonorrhoea. In the second quarter of 1979 2·5% of the number of reported cases of gonorrhoea over that period were due to PPNG infection. Because of underreporting of gonorrhoea and because a few cases of suspected and reported gonorrhoea are not confirmed by culture it is extremely difficult to calculate the exact relation between the number of cases of PPNG infection and the total number of cases of gonorrhoea in the Netherlands.

Similarly, a sudden increase in PPNG infections has been noted in Rotterdam. We observed 14 cases of gonorrhoea due to PPNG strains in the first five months of 1979 compared with 13 cases seen in the preceding 26 months. This report describes the cases seen in Rotterdam between November 1976 and June 1979.

Patients and methods

Patients who attended the venereal disease clinic of the dermatovenereology department of the University Hospital, Rotterdam, between November 1976 and June 1979 with gonorrhoea due to PPNG strains were included in the study.

DIAGNOSIS

In addition to direct microscopy of Gram-stained smears from the urethra in men and the urethra and cervix in women, routine samples for culture on Thayer-Martin media were collected from the urethra and the throat in heterosexual men and from the urethra, the cervix, the rectum, and the throat in women. All the gonococcal strains isolated in our laboratory were routinely screened for PPNG by the method previously described by Phillips *et al.*[3]

Sensitivities of the PPNG strains to tetracycline, erythromycin, spectinomycin, and cefuroxime were performed by the agar dilution method.

Auxotyping was determined by the method described by Catlin.[4] Plasmid DNA was isolated by a procedure described by Guerry and Falkow.[5]

TREATMENT

Twenty patients were treated with ampicillin or amoxycillin 3 g and probenecid 1 g orally in a single dose. Sixteen patients, including 13 patients who were still under observation, and who did not

Address for reprints: Dr E Stolz, Academisch Ziekenhuis Rotterdam, Ziekenhuis Dijkzigt, Dr Molewaterplein 40, 3015 GD Rotterdam, the Netherlands

Received for publication 12 November 1979

respond to ampicillin or amoxycillin, were treated with spectinomycin sulphate 3·2 g intramuscularly. Four patients were treated with cefuroxime 1·5 g intramuscularly.

Results

The epidemiological relationship of patients harbouring PPNG strains, their sex, profession, nationality, region of birth, and probable country of acquiring the infection are given in table I. Of the 27 cases seen in Rotterdam over the study period, 20 were in men and seven were in women. According to the history, patients 12 and 16 and patients 14 and 15 (table I) appeared to be related. Patient 23 infected a Dutch male patient, in whom only a non-PPNG strain could be isolated. However, this patient infected patient 24, who harboured a PPNG strain.[6] Thus, epidemiologically, patients 23 and 24 appeared to be related.

Of the 17 patients who acquired their infection in the Netherlands only five (four female patients and one male patient) were infected in Rotterdam. The other 12 patients were infected in The Hague and Amsterdam.

All 20 male patients were infected by prostitutes. Of the seven female patients, two were prostitutes and one was a bar-girl. Three housewives claimed to have been infected by their husbands or regular partners. One was infected by a casual Philippine friend (patient 23).

DIAGNOSIS

In the men, diagnoses were based on positive smear and culture results from the urethra in 19 cases and on a positive culture result from the urethra in one asymptomatic case. In the women, PPNG were isolated from the urethra in two cases, from the urethra and cervix in two cases, from the urethra, cervix, and rectum in one case, from the urethra, cervix, rectum, and throat in one case, and from the cervix alone in one case.

Of the 27 patients, only patients 1 and 2 acquired their infections directly in West Africa (table I). Eight patients (patients 3, 4, 5, 6, 17, 18, 22, and 23) were infected directly in South-east Asia.

TABLE I *Epidemiological data of patients harbouring PPNG strains isolated in Rotterdam*

Patient No	*Occupation*	*Country of birth*	*Infection acquired*
		West Africa	West Africa
1 (M)	Sailor	Ghana	Ghana
2 (M)	Sailor	Ivory Coast	Ivory Coast
		Northern Europe	South-east Asia
3 (M)	Sailor	Sweden	Hong Kong
4 (M)	Sailor	Norway	Thailand
5 (M)	Sailor	Norway	Philippines
		Southern Europe	
6 (M)	Sailor	Portugal	Philippines
	Immigrant (civilian)	Mediterranean	Western Europe
7 (M)	Manual worker	Morocco	The Netherlands
8 (M)	Manual worker	Spain	The Netherlands
9 (M)	Manual worker	Greece	The Netherlands
10 (M)	Manual worker	Turkey	The Netherlands
11 (M)	Student	Turkey	The Netherlands
12* (M)	Unknown	Turkey	The Netherlands
13 (M)	Unknown	Turkey	The Netherlands
14† (M)	Unknown	Morocco	The Netherlands
15† (F)	Housewife	Morocco	The Netherlands
16* (F)	Housewife	Morocco	The Netherlands
	Non-immigrant (civilian)	Western Europe	South-east Asia
17 (M)	Manual worker	The Netherlands	Thailand
18 (M)	Businessman	The Netherlands	Thailand
			Western Europe
19 (M)	Businessman	The Netherlands	The Netherlands
20 (M)	Businessman	The Netherlands	The Netherlands
			Mediterranean
21 (M)	Student	The Netherlands	Spain
			South-east Asia
22 (M)	Unknown	The Netherlands	Thailand
23‡ (F)	Air stewardess	The Netherlands	Philippines
			Western Europe
24‡ (F)	Housewife	The Netherlands	The Netherlands
25 (F)	Bar girl	The Netherlands	The Netherlands
26 (F)	Prostitute	The Netherlands	The Netherlands
27 (F)	Prostitute	The Netherlands	The Netherlands

* † ‡ Epidemiologically related

BIOLOGICAL CHARACTERISTICS OF PPNG STRAINS

The biological characteristics of PPNG strains isolated in Rotterdam are given in table II.

Non-PPNG strains were also isolated in seven patients harbouring PPNG strains; three (patients 3, 5, and 23) were infected directly in South-east Asia and three (25, 26, and 27) were highly promiscuous prostitutes. Except for patients 10 and 25, all simultaneous isolations of PPNG and non-PPNG strains in the same patient occurred in 1976 and 1977.

Auxotypes

In the patients directly infected in West Africa arginine-requiring strains were isolated whereas in the eight patients infected directly in South-east Asia proline-acquiring and zero/wild strains were isolated. In two strains no auxotyping was performed.

Plasmids

PPNG strains from patients directly infected in West Africa had a cryptic plasmid of $2\cdot5\times10^6$ daltons and a penicillinase-producing plasmid of $3\cdot3\times10^6$ daltons. No transfer plasmid was found. PPNG strains from eight patients directly infected in South-east Asia had a cryptic plasmid of $2\cdot5\times10^6$ daltons and a penicillinase-producing plasmid of $4\cdot5\times10^6$ daltons. Five of the eight PPNG strains had a larger transfer plasmid of $\pm24\times10^6$ daltons. The three patients harbouring South-east Asian PPNG strains without transfer factor were all seen in 1977.

Of the other patients not directly infected in West Africa or South-east Asia, only the two highly promiscuous prostitutes (patients 26 and 27) had auxotypes and plasmids of the West African PPNG strain-type. Neither prostitute appeared to have spread the PPNG strain she was harbouring.

Auxotypes and plasmids of the South-east Asian PPNG strain-type were found in all the other patients. A transfer plasmid could not be found in only one PPNG strain (patient 25). In the two epidemiologically related Turkish patients (12 and 16) different auxotypes were found; a comparison of plasmids was not possible in this case.

SENSITIVITIES

The PPNG isolates appeared to be less sensitive to tetracycline and cefuroxime than did other gonococcal strains isolated recently from patients in our outpatient department. Because of the small number of PPNG isolates statistical analysis has not been carried out to prove this finding. For the same reason no statistical comparison of distribution of

TABLE II *Biological characteristics of PPNG strains isolated in Rotterdam*

Patient No	*Non-PPNG strains*	*Auxotypes*	*Plasmids (daltons)*				*MICs (μg/ml) to*				*Country of direct infection*
			Cryptic $2\cdot5\times10^6$	*$3\cdot3\times10^6$ (African)*	*$4\cdot5\times10^6$ (Asian)*	*Transfer $\pm24\times10^6$*	*T*	*E*	*S*	*C*	
1 (M)		Arginine	+	+	–	–	1	4	8	ND	W Africa
2 (M)		Arginine	+	+	–	–	2	2	8	ND	W Africa
3 (M)	+	Proline	+	–	+	–	4	2	8	ND	S-E Asia
4 (M)		Proline	+	–	+	–	1	4	8	ND	S-E Asia
5 (M)	+	Proline	+	–	+	+	1	0·25	4	0·25	S-E Asia
6 (M)		Zero/Wild	+	–	+	+	1	1	8	ND	S-E Asia
7 (M)		Proline	+	–	+	+	1	1	8	0·12	S-E Asia
8 (M)		Proline	+	–	+	+	2	1	8	0·25	S-E Asia
9 (M)		Proline	+	–	+	+	4	2	8	0·25	S-E Asia
10 (M)	+	Proline	+	–	+	+	2	2	8	ND	
11 (M)		Proline	+	–	+	+	4	4	8	0·25	
12* (M)		Proline	ND	ND	ND	ND	2	1	8	0·12	
13 (M)		Proline	+	–	+	+	2	1	8	ND	
14† (M)		Proline	+	–	+	+	1	0·12	8	0·12	
15† (F)		Proline	+	–	+	+	0·25	0·12	8	0·12	
16* (F)		Zero/Wild	+	–	+	+	4	1	16	0·12	
17 (M)		ND	+	–	+	+	2	8	8	ND	S-E Asia
18 (M)		Proline	+	–	+	+	2	8	8	0·25	S-E Asia
19 (M)		Proline	+	–	+	+	2	2	8	ND	
20 (M)		Zero/Wild	+	–	+	+	2	2	8	0·25	
21 (M)		Proline	ND	ND	ND	ND	2	0·25	8	0·25	
22 (M)		ND	+	–	+	–	1	0·5	8	0·12	S-E Asia
23‡ (F)	+	Zero/Wild	+	–	+	+	1	0·25	16	ND	S-E Asia
24‡ (F)		Zero/Wild	+	–	+	+	1	0·25	16	ND	
25 (F)	+	Proline	+	–	+	–	4	1	8	0·5	
26 (F)	+	Arginine	+	+	–	–	1	2	8	ND	
27 (F)	+	Arginine	+	+	–	–	0·5	0·25	16	ND	

* † ‡ Epidemiologically related
\+ Positive – negative
ND = not done (strain died)
T = tetracycline; E = erythromycin; S = spectinomycin; C = cefuroxime

sensitivities to the four antibiotics between West African strain-type and South-east Asian strain-type isolates has been made.

Comparison of the distribution of sensitivities to erythromycin was not possible because no gonococcal strains isolated from patients in our outpatient department had been tested previously for sensitivity to this antibiotic.

All PPNG isolates were sensitive to spectinomycin (MIC between 4 and 16 μg/ml). The sensitivity patterns in the epidemiologically related strains (patients 23 and 24) were identical. A one-dilution-step difference was noted in the sensitivities to tetracycline and spectinomycin between the strains from patients 12 and 16. The two-dilution-step difference in sensitivity to tetracycline between strains from patients 14 and 15 might be explained by accepting an MIC of 0·50 μg/ml as the true MIC.

FOLLOW UP

Of the 20 patients treated with ampicillin or amoxycillin, five did not return for follow up. In 13 of the 15 cases who were available for follow up there was treatment failure; in two cases no gonococci could be isolated on the seventh day after treatment. Of the 16 patients treated with spectinomycin two did not return for follow up; treatment was successful in the remaining 14. Of the four patients treated with cefuroxime, one did not return for follow up and treatment was successful in the remaining three.

Discussion

The epidemiological data on our patients with gonorrhoea due to PPNG strains are strikingly similar to those on patients with chancroid seen in our clinic.[6] Most patients with chancroid could be classified in four main groups according to nationality: Dutch, Portuguese, Turkish, and Moroccan. Of these patients, 89% acquired their infections from prostitutes. Of the total of 53 patients, 50 were men and three were women (all of whom were prostitutes). The role of prostitutes in the spread of PPNG strains has been stressed previously[1] and has been confirmed by our data on the source of infection, as mentioned by our male patients.

Before 1977 chancroid and gonorrhoea due to PPNG strains were mostly imported by sailors and by airline personnel. It is only since 1977 that chancroid and PPNG infections have been acquired in the Netherlands. The rising incidence of chancroid in Rotterdam was noted in 1977 and 1978; the first PPNG infections in the Netherlands (Rotterdam) were recognised at the end of 1976 and the rise in incidence of cases of PPNG infection was seen in 1978 and 1979.

From an epidemiological point of view PPNG infections are behaving more like chancroid than like gonorrhoea due to non-PPNG strains. In our clinic only a few infections with gonorrhoea due to non-PPNG strains in male patients are acquired by sexual contact with prostitutes.

An important feature of our patients is that importation of PPNG strains from West Africa and South-east Asia no longer plays a major role. Apart from Dutch civilians, the most important group of patients has become the immigrant workers from the Mediterranean countries.

The isolation of both PPNG and non-PPNG strains in seven of our 27 patients may indicate plasmid instability in some PPNG strains. Such strains were isolated in the first year of our investigation more often than in the remaining period.

Interestingly, PPNG strains with the characteristics of African PPNG strains did not spread, even in those instances where highly promiscuous prostitutes were concerned. This may be due to the instability of the penicillinase-producing plasmid involved or to the fact that these African strains lack a larger "stabilising" transfer plasmid of 24×10^6 daltons.

As far as conclusions can be drawn from our limited material in Rotterdam, mainly plasmid-stable PPNG strains with characteristics of PPNG strains from the Philippines containing transfer plasmids are circulating in the Netherlands. The fact that these strains seem to be less sensitive to tetracycline and cefuroxime than non-PPNG strains isolated in Rotterdam is alarming. PPNG strains which are less sensitive to several antibiotics have been reported previously—namely, those isolated in the Philippines and South-east Asia.[1]

Auxotyping and plasmid identifications seem to be useful in studying the epidemiology of PPNG infections, as has been shown by this study. Additional studies of sensitivity are necessary to maintain or to alter existing treatment schedules. In our study all PPNG isolates were sensitive to spectinomycin, and spectinomycin sulphate 3·2 g intramuscularly proved to be effective in treating patients with infections due to PPNG strains. The number of patients treated with cefuroxime was too small to evaluate the results.

We wish to thank Dr J D A van Embden of the National Institute of Public Health in Bilthoven and Mrs G Bosscher-Koetsier, Mrs M de Jonge-Suy, Mrs S de Weerdt-van Ameyden, and Mr E O'Niel of the Routine Laboratory of the University Hospital Rotterdam-Dijkzigt.

References

1. Perine PL, Morton RS, Piot P, Siegel MS, Antal GM. Epidemiology and treatment of penicillinase-producing *Neisseria gonorrhoeae*. *WHO/VDT/79.419. WHO/VDT/RES/GON/79.122.*
2. Arya OP, Rees E, Percival A, Alergant CD, Annels EH, Turner GG. Epidemiology and treatment of gonorrhoea caused by penicillinase-producing strains in Liverpool. *Br J Vener Dis* 1978;**54**:28-35.
3. Phillips CW, Aller RC, Cohen CN: Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;ii:960.
4. Catlin WB. Nutritional profiles of *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. *J Infect Dis* 1973;**128**:178-94.
5. Guerry PD, LeBlanc DJ. Falkow S. General method for isolation of plasmid deoxyribonucleic acid. *J Bact* 1973;**116**:1064-6.
6. Nayyar KC, Stolz E, Michel MF. Rising incidence of chancroid in Rotterdam. Epidemiological, clinical, diagnostic and therapeutic aspects. *Br J Vener Dis* 1979;**55**:439-41.

Br J Vener Dis 1980;56:249-51.

Antibiotic sensitivities of gonococci isolated in Rotterdam and results of treatment with cefuroxime

K C NAYYAR,* M F MICHEL,† AND E STOLZ*

*From the Departments of *Dermatology and †Microbiology, University Hospital Rotterdam-Dijkzigt, Rotterdam, the Netherlands*

SUMMARY In a study of the efficacy of cefuroxime in the treatment of 278 cases of acute gonorrhoea (including rectal and pharyngeal infections and infections due to penicillinase-producing *Neisseria gonorrhoeae*), high cure rates were found for both male (98·8%) and female patients (98·6%). Disadvantages of this treatment are its high cost and the relatively high proportion of patients who develop postgonococcal urethritis.

Introduction

Penicillin has been the drug of choice in the treatment of gonorrhoea ever since its discovery. However, in view of the emergence of penicillinase-producing strains of *Neisseria gonorrhoeae* (PPNG), it has become necessary to reassess the role of penicillins and ampicillins in the treatment of gonorrhoea. In the Netherlands infections caused by PPNG strains are becoming a serious problem. In the second quarter of 1979 about 2500 cases of gonorrhoea, and about 60 (2·5%) cases caused by PPNG strains, were reported to the Inspector of Health.[1]

The continued use of penicillins and ampicillins as drugs of first choice will probably cause several PPNG strains to become resistant to the drugs and also an unnecessary delay in the effective treatment of patients with infections due to PPNG strains. From an epidemiological point of view this is highly undesirable and theoretically a further increase in the proportion of infections due to PPNG strains can be expected. At a time when amoxycillin 3 g and probenecid 1 g orally was standard treatment in our clinic five (25%) out of 20 patients harbouring PPNG strains could not be followed up. Of the remaining 15 patients, gonococci could not be isolated in two one week after treatment. The other 13 patients still harboured PPNG strains 4-7 days after treatment.[1]

For this reason it became necessary for us to look for alternative treatments. β-lactamase-resistant cephalosporins are drugs which can be used in infections due to PPNG strains.[2]

Recently, Berg *et al*[3] investigated the efficacy of cefoxitin in cases of gonorrhoea due to PPNG as well as to non-PPNG strains. In both types of infection treatment with cefoxitin was completely successful. One of the other promising β-lactamase-resistant cephalosporins is cefuroxime, a semisynthetic analogue of cephalosporin C; it has a broad-spectrum activity, is not absorbed orally, and has to be administered parenterally.

In this study, the efficacy of cefuroxime (1·5 g administered in a single intramuscular injection) in the routine treatment of uncomplicated urogenital, rectal, and pharyngeal gonorrhoea due to non-PPNG and PPNG strains is evaluated.

Patients and methods

Patients diagnosed as having gonorrhoea in the venereal disease clinic of the dermatovenereology department of the University Hospital Rotterdam-Dijkzigt between June 1979 and August 1979 were included in this study. Sailors, prostitutes, pregnant women, and patients with a history of penicillin allergy were not included. A total of 278 patients (197 men and 81 women) with urogenital, rectal, or pharyngeal gonorrhoea were treated.

DIAGNOSIS

The diagnosis of gonorrhoea in these patients was based on a positive result to culture for *N gonorrhoeae*. The diagnostic procedure in these patients was performed as described previously.[4]

All strains were screened for penicillinase production by the phenol red method.[5]

DRUG SENSITIVITIES

Of the first 157 strains isolated (116 strains from male and 41 strains from female patients) sensitivities to

Address for reprints: Dr K C Nayyar, Department of Dermatology, University Hospital Rotterdam-Dijkzigt, Rotterdam, the Netherlands

Received for publication 5 November 1979

amoxycillin, penicillin, tetracycline, and cefuroxime were determined by the agar dilution method as previously described.[6] The amoxycillin, penicillin, tetracycline, and cefuroxime concentrations were varied in doubling dilutions over the following ranges: 0·0025-2·56 μg/ml (amoxycillin and penicillin); 0·01-2·56 μg/ml (tetracycline); and 0·0025-0·64 μg/ml (cefuroxime). Sensitivities were expressed as minimum inhibitory concentrations (MICs) in μg/ml. Relative resistance was arbitrarily defined as follows: amoxycillin, MIC >0·16 μg/ml; penicillin, MIC >0·08 μg/ml; tetracycline, MIC >1·28 μg/ml; and cefuroxime, MIC >0·04 μg/ml. Strains having an MIC >2·56 μg/ml for amoxycillin and penicillin were considered to be resistant.

FOLLOW UP

The patients were asked to return for review 7-11 days after treatment. At review, examination was the same as that performed before treatment. Any patient found to have gonorrhoea during the 11 days after treatment, and who admitted to new sexual contact within this period, was regarded as a case of reinfection. If the patient denied having had any sexual contact in this period and still had gonorrhoea, this was regarded as a treatment failure.

The male patients were investigated for post-gonococcal urethritis (PGU) on the basis of the sedimentation results in the two-glass urine test (>10 pus cells/high power field (×25).

Results

DIAGNOSIS

In the male patients, the diagnosis of gonorrhoea was based on positive culture results from the urethra in 190, from the urethra and throat in two, and from the rectum in five. In the female patients, the diagnosis was based on positive culture results from the urethra in one, from the cervix in six, from the rectum in one, from the cervix and urethra in 50, from the cervix and rectum in four, from the cervix, urethra, and rectum in 16, from the cervix, urethra, and throat in two, and from the cervix, urethra, rectum, and throat in one. Eleven (4%) patients (seven (3·6%) male and four (4·9%) female) harboured PPNG strains. Of the 11 PPNG strains, 10 (six from male and four from female patients) were included in the 157 strains tested for sensitivity to the four antibiotics mentioned earlier.

DRUG SENSITIVITIES

The distribution of the sensitivities and the percentage of (relative) resistance to amoxycillin, penicillin, tetracycline, and cefuroxime are given in table I. All the 10 PPNG strains had an MIC >2·56 μg/ml for amoxycillin and penicillin. Nine of these 10 strains were tested for sensitivity to cefuroxime and tetracycline. All had an MIC of 0·16 μg/ml for cefuroxime. The MIC of tetracycline was 2·56 μg/ml for one strain and 0·64 μg/ml for eight strains.

TABLE *Sensitivities of 157 gonococcal strains to amoxycillin, penicillin, tetracycline, and cefuroxime and percentage with relative resistance to the four drugs*

	% of gonococcal strains with MICs (μg/ml) as follows:													
Drug	*0·0025*	*0·005*	*0·01*	*0·02*	*0·04*	*0·08*	*0·16*	*0·32*	*0·64*	*1·28*	*2·56*	*>2·56**	*Total No*	*% with relative resistance†*
Amoxycillin														
M‡			4	13	19	8	29	17	4	0		5	116	56
F‡			0	7	32	12	27	10	0	2		10	41	49
M+F			3	11	22	9	29	15	3	1		6	157	54
Penicillin														
M	1	9	18	9	9	25	11	6	6	0		5	116	53
F	0	0	22	22	10	22	5	2	5	0	2	10	4	46
M+F	1	6	19	13	10	24	10	5	6	0	1	6	157	52
Tetracycline														
M				1	0	0	10	47	10	23	10		115	33
F				0	0	2	5	54	10	17	12		41	29
M+F				1	0	1	8	49	10	21	11		156	32
Cefuroxime														
M		8	20	28	16	11	15	3					115§	44
F		0	24	34	15	5	22	0					41	41
M+F		6	21	29	15	10	17	2					156	44

* Penicillinase-producing *N gonorrhoeae* (PPNG) strains
† To amoxycillin, MIC >0·16 μg/ml; to penicillin, MIC >0·08 μg/ml; to tetracycline, MIC >1·28 μg/ml; to cefuroxime, MIC >0·04 μg/ml
‡ Strains isolated from male patients (M) and female patients (F)
§ One strain not tested

FOLLOW UP

Of the 267 patients infected with non-PPNG strains (190 male and 77 female), 20 (7·5%) patients (15 male, 7·9% and five female, 6·5%) did not return for follow up. Of the remaining 175 male patients, 173 (98·8%) were cured. One (0·6%) patient admitted reinfection; another (0·6%) was not cured at the first review one week after treatment. The MIC of cefuroxime for the isolated strains was not determined. Forty-seven (26·9%) patients developed postgonococcal urethritis. Of the 72 female patients who did return, 71 (98·6%) were cured. One (1·4%) patient was still infected one week after treatment; she admitted re-exposure to infection.

Of all the 11 patients infected with PPNG strains (seven male and four female) one male patient was not available for follow up; the remaining 10 patients were all cured, as were all patients with rectal or pharyngeal gonorrhoea.

No serious side effects were observed. Two patients developed an erythematous maculopapular rash. Twenty-one patients did complain of transient pain at the injection site.

Discussion

The prevalence of PPNG strains in our study population (4%) corresponds with that in the Netherlands during the second quarter of 1979 (2·5%).

The proportion of relatively resistant strains to amoxycillin, penicillin, tetracycline, and cefuroxime was higher in this study than in those performed in 1976 and between 1971 and 1972 of gonococci isolated from male and female civilian patients attending our Rotterdam outpatient department.[6 7] Even when the PPNG strains are excluded, the proportion of relatively resistant strains to amoxycillin, penicillin, tetracycline, and cefuroxime are still higher in this study than in the earlier studies (table II).

The relative resistance to amoxycillin and tetracycline has markedly increased. Amoxycillin is used as the treatment of choice in our clinic and elsewhere in the Netherlands, and tetracycline is sometimes used for patients with gonorrhoea. Our findings need to be corroborated by larger scale studies testing sensitivities to a broader range of antibiotics in our clinic as well as in other European centres.

Only one of the PPNG strains tested was relatively resistant to tetracycline. The MICs for cefuroxime were also not very high (0·16 μg/ml).

Despite a small increase in the incidence of relative resistance to cefuroxime in only one male patient, treatment failure with cefuroxime was noted. According to Fowler and Rahim,[8] the proportion of patients developing postgonococcal urethritis after treatment with cefuroxime is similar to that after treatment with penicillin or ampicillin. This is confirmed by our data.

The results of this study show that cefuroxime 1·5 g in a single intramuscular dose is safe and highly effective in the treatment of gonorrhoea whether or not the patient is harbouring a PPNG strain. One drawback of treatment with cefuroxime, as well as with ampicillin and penicillin, is the high incidence of postgonococcal urethritis in male patients. Another disadvantage is the high cost of treatment with cefuroxime in the Netherlands; treatment with cefuroxime costs 75% more than with amoxycillin and probenecid. Thus, the advantages and the disadvantages of treatment with cefuroxime have to be considered before cefuroxime is used as a drug of first choice in the treatment of gonorrhoea. This decision will depend on the increased prevalence of PPNG strains, on the availability of other first-line drugs for treating uncomplicated gonorrhoea, on the possibility that gonococci will rapidly develop resistance to cefuroxime in vitro and in vivo, and on the costs involved in a change of drug regimen.

TABLE II *Percentage of relatively resistant strains to amoxycillin, penicillin, tetracycline, and cefuroxime for gonococcal strains isolated in 1971/2, 1976, and 1979*

	% of strains relatively resistant			
	Non-PPNG			*Non-PPNG + PPNG*
Drug	*1971/2 (n = 430)*	*1976 (n = 146)*	*1979 (n = 147)*	*1979 (n = 157)*
Amoxycillin	38	31	51	54
Penicillin	37	40	48	52
Tetracycline	15	8	29	32
Cefuroxime		35	40	44

References

1. Nayyar KC, Noble RC, Michel MF, Stolz E. Gonorrhoea in Rotterdam caused by penicillinase-producing gonococci. *Br J Vener Dis* 1980;**56**:244-8.
2. O'Callaghan CH, Sykes RB, Griffiths A, Thornton JE. Cefuroxime a new cephalosporin antibiotic activity in vitro. *Antimicrob Agent Chemother* 1976;**9**:511-9.
3. Berg SW, Kilpatrick ME, Harrison WO, McCutchan JA. Cefoxitin treatment of penicillinase-producing *Neisseria gonorrhoeae*. *N Engl J Med* 1979;**301**:509-11.
4. Stolz E. Diagnostic aspects of gonorrhoea. *Thesis*. Rotterdam, 1974.
5. Phillips CW, Aller RC, Cohen CN. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;ii:960.
6. Stolz E, Zwart HGF, Michel MF. Sensitivity to ampicillin, penicillin and tetracycline of gonococci in Rotterdam. *Br J Vener Dis* 1974;**50**:202-7.
7. Stolz E, Michel MF, Schuller JL, de Koning GAJ. Sensitivity to gonococcus strains isolated in Rotterdam to epicillin, ampicillin, penicillin and tetracycline. *Dermatologica* 1978;**157**:158-63.
8. Fowler W, Rahim G. Cefuroxime and postgonococcal urethritis. *Roy Soc Med* 1977;**70** suppl 90:131-3.

Br J Vener Dis 1980;**56**:252-4.

Sensitivity of Neisseria gonorrhoeae to spectinomycin and thiamphenicol

K GRUNDER AND D PETZOLDT

From the Department of Dermatology and Venereology, University Medical School, Lübeck, West Germany

SUMMARY Between 1966 and 1978 the sensitivities of strains of *Neisseria gonorrhoeae* (70-80 strains in each year) to penicillin, thiamphenicol, and spectinomycin were tested. For penicillin the proportion of less sensitive strains increased from 21% in 1976 to 41% in 1978 and for thiamphenicol from 10% to 18% over the same period. All the strains proved to be sensitive to spectinomycin. A significant correlation in the degree of sensitivity was observed between penicillin and thiamphenicol but not between penicillin and spectinomycin.

Introduction

All over the world strains of *Neisseria gonorrhoeae* have shown a steady reduction in sensitivity to penicillin. This situation demands the continued development of suitable alternatives for the treatment of gonorrhoea.

In 1976 in Lübeck the situation was relatively favourable with only 21% less sensitive strains (IC_{50} 0·063 IU/ml) (fig 1).[1] In 1977 this proportion increased to 31% and in 1978 to 41%; the graph shows the higher peaks moving to the right (fig 1). The increase in mean 50% concentration (IC_{50}) from 0·08 IU/ml to 0·19 IU/ml is significant ($t = 3{\cdot}4732$; $P < 0{\cdot}001$). Consequently, conditions here are now similar to those observed in other regions some years ago.[2 3]

Although the so-called less sensitive strains can still be treated with larger doses of penicillin, the limit of effectiveness of penicillin will be reached in a few years' time if this trend persists. In addition, there have been reports from a number of countries in the last three years of β-lactamase-producing strains with an absolute resistance to penicillin.[4-15]

For these reasons we have tested the sensitivity of strains isolated in the Lübeck area (preserved since 1976 in liquid nitrogen) to spectinomycin and thiamphenicol and compared the results with their sensitivity to penicillin.

Materials and methods

The sensitivity of strains to spectinomycin and thiamphenicol was determined by the plate dilution method according to the directions of the WHO International Reference Centre for Gonococci in Copenhagen.[16] The evaluation was performed by the Kärber method and a mean IC_{50} was calculated.[16]

STATISTICAL ANALYSIS

Student's *t* test was used to compare differences of antibiotic sensitivity in the different years. Where correlations between two antibiotics were obvious we used the non-parametric graphical medial test, as described by Quenouille;[17] where correlations were doubtful a rank correlation method with ties (Spearman) was performed.[18]

Results

The sensitivity of the strains to spectinomycin and thiamphenicol over the three-year period is shown in fig 2. Between 1976 and 1978 the mean IC_{50} of spectinomycin increased slightly but significantly from 7 μg/ml to 8·5 μg/ml ($t = 3{\cdot}6608$; $P < 0{\cdot}001$). Although the peak remained at 10 μg/ml the percentage of strains sensitive to 10 μg/ml increased. None of the strains, including a β-lactamase-producing strain imported from Manila and examined in our laboratory, exceeded the limit of 10 μg/ml. Therefore all the strains must be regarded as highly sensitive. The asymmetrical distribution with the maximum peaks at the right side corresponds with the results of other studies.[19-23]

The mean IC_{50} of thiamphenicol increased significantly from 0·21 μg/ml to 0·34 μg/ml ($t = 4{\cdot}4188$; $P < 0{\cdot}001$); the peak shifted to the right approximately according to the penicillin distribution. The proportion of less sensitive strains increased from 10% in 1976 to 18% in 1978. In 1978, two strains had an IC_{50} of 1·41 μg/ml and must be

Address for reprints: Professor D Petzoldt, Universitäts Hautklinik, Voßtrasse 2, D-6900 Heidelberg, West Germany

Received for publication 24 September 1979

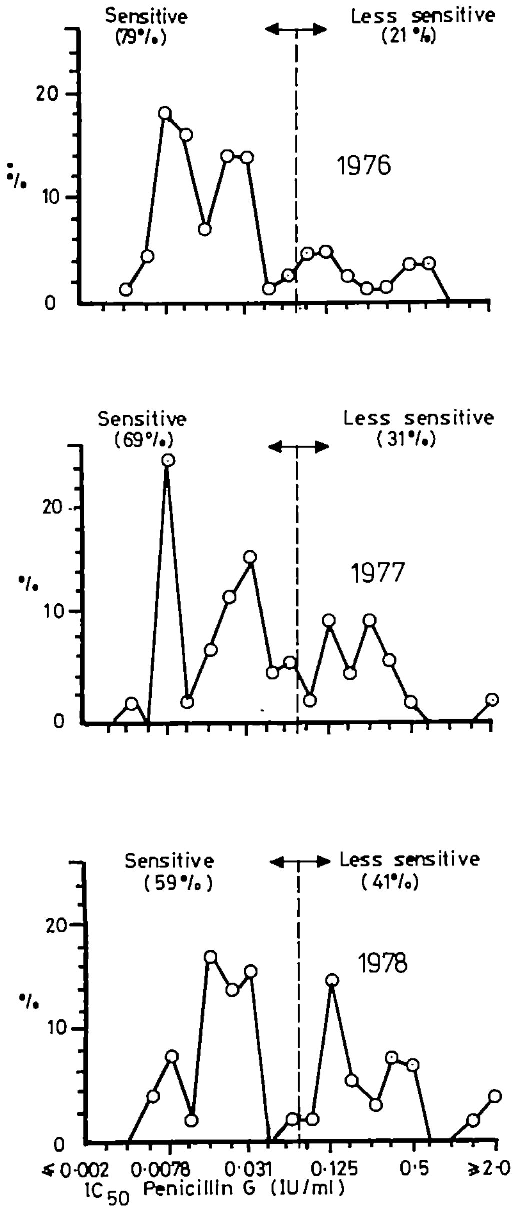


FIG 1 *Sensitivity of strains of N gonorrhoeae to penicillin in the Lübeck area in 1976, 1977, and 1978.*

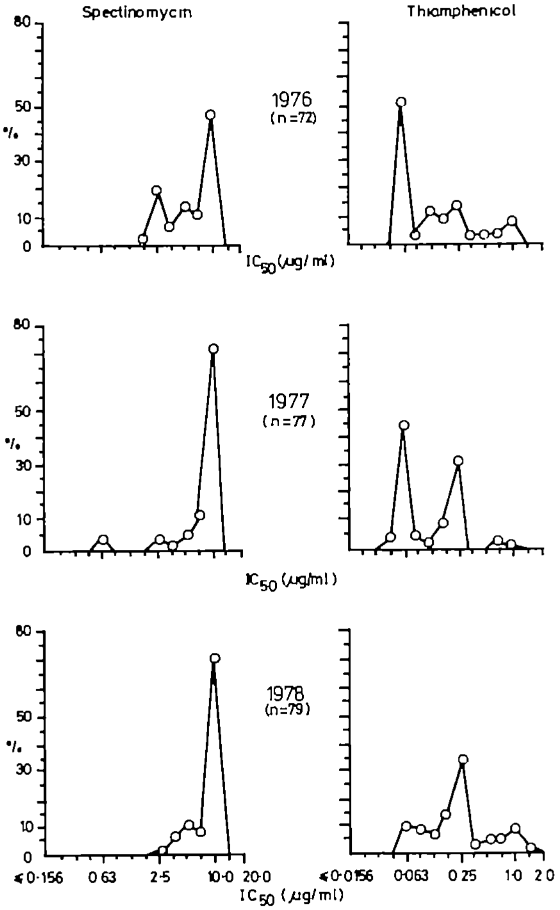


FIG 2 *Sensitivity of strains of N gonorrhoeae to spectinomycin and thiamphenicol in the Lübeck area in 1976, 1977, and 1978.*

regarded as resistant (one of them was the β-lactamase-producing strain mentioned above).

The correlation of spectinomycin and thiamphenicol sensitivities with those of penicillin for all strains isolated in the three years with 228 pairs of observations are shown in fig 3. The correlation with penicillin was not significant for spectinomycin but was significant for thiamphenicol, as also reported by other authors.[20] Although the regression line on the graph has visual impact, it is not quite correct since the values are not normally distributed. In the statistical evaluation of the correlation between penicillin and thiamphenicol we therefore used the non-parametric graphical medial test and found the correlation was significant at the 1% level.

The correlation between penicillin and spectinomycin proved not to be significant by Spearman's rank correlation with ties ($r_S^* = 0{\cdot}024$; $2\alpha > 0{\cdot}1$).

Discussion

Our investigation of the sensitivities of strains of *N gonorrhoeae* to two alternative antibiotics showed that both were suitable in cases where penicillin treatment was unsuccessful or, for other reasons, impossible.

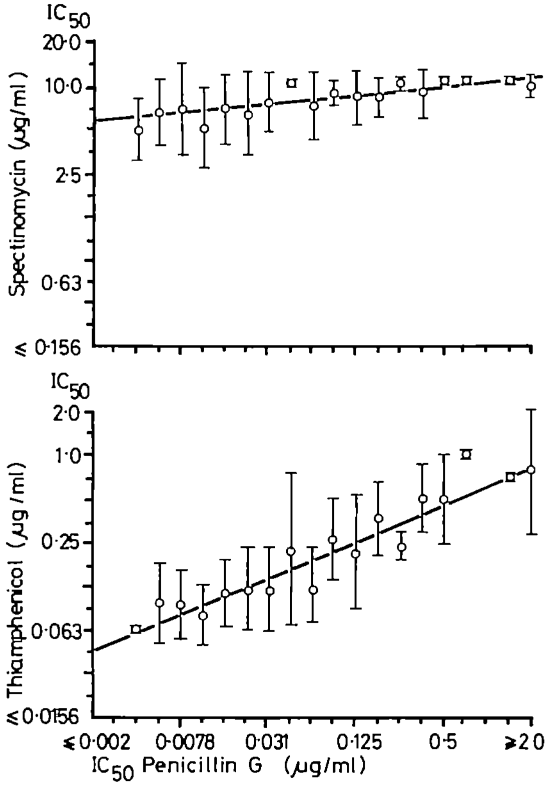


FIG 3 *Correlation of sensitivities to spectinomycin and thiamphenicol with those to penicillin showing mean values with their standard deviation.*

Sensitivity to thiamphenicol was significantly correlated with that to penicillin, but the proportion of less sensitive strains was significantly smaller—8% for thiamphenicol compared with 41% for penicillin in 1978.

In an appraisal of spectinomycin the following points should be considered:

(1) An increase of mean IC_{50} occurred during the three-year period, but it was insignificant.

(2) The proportion of less sensitive strains did not increase.

(3) The margin of therapeutic action is still considerable, assuming that the breakpoint for sensitive bacteria is 30 μg/ml.

(4) Because the correlation with penicillin sensitivity was insignificant a marked change in the present favourable situation should not be expected in the near future.

Periodic investigation of sensitivity to spectinomycin is indicated, particularly to recognise an increasing resistance of the "one-step" type.

References

1. Gründer K, Petzoldt D. Die penicillinempfindlichkeit von *Neisseria gonorrhoeae* im Lübecker Raum unter besonderer berücksichtigung etwaiger penicillinasebildner. *Hautarzt* 1977;**28** suppl 2:87-90.
2. Petzoldt D, Reyn A. Die penicillinempfindlichkeit von *Neisseria gonorrhoeae* im Münchner Raum. *Z Hautkr* 1976;**51** suppl 2:115-9.
3. Petzoldt D, Gründer K, Neubert U. Sensitivity of *N gonorrhoeae* to penicillin in West Germany. *Br J Vener Dis* 1979;**55**:80-2.
4. Arya OP, Rees E, Percival A, Alergant CD, Annels EH, Turner GC. Some clinical-epidemiological facts of gonorrhoea due to penicillinase-producing strains in Merseyside. *Br J Vener Dis* 1978;**54**:28-35.
5. Arya OP, Percival A. Outbreak of gonorrhoea due to β-lactamase-producing gonococci in Liverpool: a short report. *WHO/VDT/77415* 1977;1-7.
6. Ashford WA, Golash RG, Hemming VG. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;ii:657-8.
7. Hallet AF, Appelbaum PC, Cooper R, Mokgokong S, Monale D. Penicillinase-producing *Neisseria gonorrhoeae* from South Africa. *Lancet* 1977;i:1205.
8. Milatović D, Machka K, Galla O, Braveny I. β-lactamase bildende gonokokken in München. *Infection* 1978;**6**:242-3.
9. Neubert U, Ruckdeschel G. Isolierung penicillinasebildender gonokokken in München. *Münch Med Wochenschr* 1978;**120**:1063-4.
10. Percival A, Corkill JE, Arya OP, Rowlands J, Alergant CD, Rees E. Penicillinase-producing gonococci in Liverpool. *Lancet* 1976;ii:1379-82.
11. Philips CW, Aller RD, Cohen NS. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;ii:960.
12. Philips J. β-lactamase-producing, penicillin-resistant gonococcus. *Lancet* 1976;ii:656-7.
13. Turner GC, Ratcliffe JG, Anderson D. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;ii:793.
14. Wilkinson AE, Seth AD, Rodin P. Infection with penicillinase-producing gonococcus. *Br Med J* 1976;ii:1233.
15. Gründer K, Petzoldt D, Schoop HJ, Eidmann E. Erneutes auftreten penicillinasebildender *Neisseria gonorrhoeae* in Deutschland. *Z Hautkr* 1979;**54**:465-8.
16. Reyn A, Weis Bentzon M, Ericsson H. Comparative investigations of the sensitivity of *N gonorrhoeae* to penicillin. *Acta Path Microbiol Scand* 1963;**57**:235-55.
17. Quenouille MH. *Associated Measurements*. London: Butterworth Press, 1952:37-40.
18. Kendall MG. *Rank Correlation Methods*, 3rd ed. London 1962:38-41.
19. Dillon JR, Eidus L, Diena BB. Cross-Canada survey of susceptibility of *Neisseria gonorrhoeae* to six antimicrobial agents. *Can Med Assoc J* 1978;**119**:223-8.
20. Füllhaas I, Vischer WA, Brügger P, Rufli T. Antibiotic sensitivity of gonococcal strains isolated from patients in Basel and environs. *Dermatologica* 1979;**158**:249-56.
21. Levy I, Wicher K, Rose NR. In-vitro susceptibility of *Neisseria gonorrhoeae* to spectinomycin examined by a broth dilution method. *Antimicrob Agents Chemother* 1973;**3**:335-7.
22. Meheus A, Piot P, Patty S, van Dyck E, Vanden Berghe D. Activity in vitro of 10 antimicrobial agents against *Neisseria gonorrhoeae*. A study of the correlation between the sensitivities. *Br J Vener Dis* 1976;**52**:329-32.
23. Meyer-Rohn J. Resistenzsituation von *Neisseria gonorrhoeae* gegenüber spectinomycin. *Z Hautkr* 1977;**52** suppl 1:7-8.

Br J Vener Dis 1980;**56**:255-8.

HR 756—a new cephalosporin in the treatment of gonorrhoea caused by ordinary and penicillinase-producing strains of Neisseria gonorrhoeae

V S RAJAN,* E H SNG,† ROGER PANG,* N J TAN,* T THIRUMOORTHY,* AND K L YEO†

*From the *Middle Road Hospital, and the †Department of Pathology, Singapore General Hospital, Singapore*

SUMMARY HR 756, a new cephalosporin, was used in single intramuscular doses of 500 mg to treat 108 men and women with gonorrhoea caused by penicillinase-producing *Neisseria gonorrhoeae* (PPNG) and non-PPNG. Of 102 patients followed up, 99 (97·05%) were cured. Cure rates for PPNG infections and non-PPNG infections were 98·18% and 95·74% respectively. Few adverse side effects were recorded but possible cross-sensitisation with penicillin was observed. Clinical and laboratory antibiotic susceptibility results correlated well. It is concluded that this drug is safe and effective in treating both PPNG and non-PPNG infections.

Introduction

Gonococci are developing partial and total resistance to penicillin. In late 1975, β-lactamase-producing, or penicillinase-producing, *Neisseria gonorrhoeae* (PPNG) emerged and in the following years quickly found a foothold in certain African and Asian countries. There is thus a need to search for effective alternatives to penicillin in combating these gonococci. None of the alternative drugs listed by the World Health Organisation have the same advantages as penicillin, since some are costly, others toxic, or they may need to be given in multiple doses.[1] The cephalosporins were recognised early to have potential in treating gonorrhoea. However, they and their analogues gave poor treatment results.[2-4] Changes in the chemical structure of the cephalosporins have resulted in an increased antibacterial spectrum, more useful pharmacokinetic properties, and, in some cases, a high level of resistance to β-lactamase hydrolysis.

A second generation cephalosporin, HR 756, 7-2-(2-amino-4-thiazolyl)-2-(Z)-(methoximino) acetamidocephalosporanic acid (figure) has been found to have great β-lactamase stability.[5] We have carried out a study to determine the efficacy of HR 756 in the treatment of gonorrhoea caused by the PPNG and non-PPNG strains. The drug used was cefotaxime, a water-soluble sodium salt form of HR 756. The clinical response was correlated with antibiotic susceptibility determined in the laboratory.

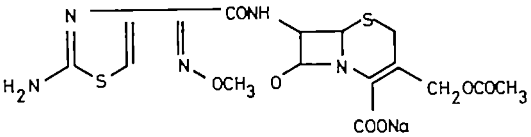


FIG *HR 756, 7-2-(2-amino-4-thiazolyl)-2-(Z)-(methoximino)acetamidocephalosporanic acid*

Patients and methods

STUDY POPULATION

Each patient was given a single intramuscular injection of HR 756 together with probenecid 1 g orally. The following groups of patients were studied:

Group A. This consisted of male and female prostitutes who came to the hospital for regular check-up. Treatment was given to women with positive results to cervical or rectal cultures of PPNG strains only whereas all men with positive rectal results (PPNG and non-PPNG) were treated.

Group B. This consisted of other patients (male and female) who attended the hospital. Treatment was given to those with positive results to urethral, cervical, or rectal smears (showing intracellular Gram-negative diplococci). Specimens were taken at the same time for culture for *N gonorrhoeae*. Only those treated patients with confirmed positive culture results were included in the study.

Address for reprints: Dr V S Rajan, Middle Road Hospital, Singapore 7, Republic of Singapore

Received for publication 21 November 1979

PRETREATMENT INVESTIGATIONS

Before treatment, the following information was recorded: presence of other sexually transmitted diseases, concomitant medication during the period of study, and the body weight of the patient. The following blood tests were also performed: total white and differential counts, platelet counts, blood SGPT, and urea. These were performed because the drug manufacturer reported a low percentage (0·3-0·9%) of leucopenia, thrombocytopenia, eosinophilia, and elevated blood SGPT and urea in some patients given HR 756 for other conditions. The blood tests were repeated on the seventh and fourteenth day after treatment.

TESTS-OF-CURE

Tests of cure were determined by cultures carried out on the third, seventh, and fourteenth day.

Patients in group B were considered to be cured of the infection when follow-up cultures gave negative results. Treatment failure was diagnosed when one of the cultures gave a positive result and the possibility of reinfection was excluded. Patients in group A usually did not refrain from sexual activities for more than three days. The criteria for cure in this group were therefore modified. A patient was considered cured if cultures continued to give negative results for one week after treatment. When cultures gave positive results on the seventh or fourteenth day, reinfection was diagnosed if patients had resumed sexual activities, otherwise they were considered to be treatment failures.

For cultures, all swabs were inoculated directly on to modified Thayer-Martin medium and incubated at 35°C for 24-48 hours in CO_2 canisters.[6] Identification was by colonial morphology, oxidase test, Gram staining and sugar utilisation. All cultures giving positive results were tested routinely for the presence of penicillinase by the disc diffusion technique using a penicillin disc (10 IU) and confirmed by the rapid iodometric method.[7]

ANTIBIOTIC SUSCEPTIBILITY

Antibiotic susceptibility was determined routinely in isolates giving positive results. The minimum inhibitory concentrations (MICs) of HR 756 and penicillin G were determined by the agar plate dilution method. Two-fold concentrations of HR 756 from 0·00025 to 0·03 μg/ml and penicillin G from 0·016 to 4 μg/ml were distributed into GC agar base (Difco) supplemented with haemoglobin and IsoVitalex. The test organisms were grown on chocolate agar and then suspended in Mueller-Hinton broth. The suspension turbidity was adjusted to correspond to 0·5 McFarland standard barium sulphate. The suspension was further diluted 1/100, and 10 μl of each suspension was inoculated on to the antibiotic-containing medium using a multi-point inoculator. The plates were incubated in CO_2 canisters at 35°C overnight. The MIC was determined as the lowest concentration of antibiotic that permitted the growth of no more than one colony.

STATISTICAL ANALYSIS

Student's *t* test was used to determine the significance of the difference between the mean MICs of PPNG and non-PPNG strains. For the correlation between the MICs of HR 756 and penicillin the product moment method was used.

Results

One hundred and eight patients with bacteriologically confirmed gonorrhoea were treated with HR 756. Of these infections, 57 were due to PPNG and 51 to non-PPNG (table I).

TABLE I *Number of PPNG and non-PPNG infections in patients studied*

	Group A	*Group B*	*Total*
PPNG	44	13	57
Non-PPNG	10	41	51
Total	54	54	108

FOLLOW UP

Details of follow-up attendances of patients after treatment are shown in table II.

TREATMENT RESPONSE

Of 102 patients followed up, 99 (97·05%) were cured and three (2·94%) failed to respond to treatment (table III). Of the 55 patients with PPNG infections who attended for follow up, 54 (98·18%) were cured. Similarly, 45 (95·74%) out of 47 patients with non-PPNG infections responded to treatment. Note that all 11 male prostitutes with positive rectal culture results were cured of their infections. There were four reinfections in group A and none in group B.

ANTIBIOTIC SUSCEPTIBILITY

Sixty-eight strains were tested for their antibiotic susceptibilities. All the strains were susceptible to 0·03 μg/ml of HR 756 (table IV). The mean MICs for PPNG and non-PPNG strains were 0·11 and 0·0045 μg/ml respectively. This difference is statistically significant ($t = 3{\cdot}3140$; $P < 0{\cdot}01$).

TABLE II *Follow up of patients after treatment*

Attendance after treatment	*Group A*	*Group B*	*Total*
On 3rd and 7th day	0	9	9
On 3rd, 7th and 14th day	53	40	93
Defaulted	1	5	6
Total	54	54	108

TABLE III *Results of treatment*

Results of treatment	*Strains*	*Group A*		*Group B*		*Total (%)*		
		M	*F*	*M*	*F*			
Success	PPNG	3	39	10	2	54	99 (97·05)	102 (100)
	Non-PPNG	8	2	35	0	45		
Failure	PPNG	0	1	0	0	1	3 (2·94)	
	Non-PPNG	0	0	2	0	2		
Defaulted	PPNG	0	1	1	0	2	6	
	Non-PPNG	0	0	4	0	4		

All the PPNG strains grew in the presence of 4 μg/ml of penicillin G. The mean MIC of penicillin G for the non-PPNG strains was 0·5 μg/ml. This is higher than the amount of HR 756 needed to inhibit either PPNG or non-PPNG strains. The concentration of HR 756 needed to inhibit non-PPNG strains was directly related to the MIC of penicillin G for these strains (table V). The correlation is statistically significant ($t = 6{\cdot}6156$; $P < 0{\cdot}001$).

The MICs of HR 756 for strains from the three patients who failed to respond to treatment were 0·016, 0·008, and 0·008 μg/ml. When these are compared with those for the other strains tested, the treatment failure rates with different MICs may be found (table VI); treatment failure is apparent when the MIC is 0·008 μg/ml or above.

DRUG TOLERANCE

Pain at injection site. Two patients complained of severe pain for one hour and 11 of moderate pain for half to one hour. The others found little discomfort after injection.

Local reactions. None was recorded.

Systemic reactions. Two patients complained of fever and giddiness a few hours after injection and this lasted for about 12 hours. One patient complained of chills and rigor five hours after injection, which was followed by fever for one day. This particular patient was known to be hypersensitive to penicillin. Another patient complained of generalised skin irritation for one week but there was no obvious rash.

Blood counts and biochemical tests. Blood counts, blood SGPT, and urea concentrations before and after treatment showed no significant change.

Effect of concomitant medication. Sixteen female patients were taking oral contraceptives, five male prostitutes were taking oestrogen pills, and one male patient was taking antacids for gastric discomfort. All of them responded to treatment.

Discussion

The early cephalosporins were not very effective against *N gonorrhoeae*, but some of the newer ones, cephamandole, cefuroxime, and cefoxitin (cephamycin), are found to be effective against both PPNG and non-PPNG strains.[8-10] HR 756 is one of the second generation of cephalosporins which is highly effective against a wide variety of Gram-positive and Gram-negative bacteria, including *N gonorrhoeae.*[11-13]

In the present study, the clinical response was very good. The overall cure rate was 97·05% while that of PPNG and non-PPNG infections was 98·2% and 95·7% respectively. These results are totally acceptable epidemiologically. Bacteriologically, it was found that all the strains were inhibited by 0·3 μg/ml of the antibiotic. This is comparable to a previous study, in which 0·016 μg/ml of the antibiotic inhibited 68 strains of PPNG and 69 strains of non-PPNG.[13] It has been reported that higher concentrations of HR 756 are needed to inhibit non-PPNG strains, which were less susceptible to penicillin. This is confirmed in the present study, in which the MICs of HR 756 correlated directly with those of penicillin.

In-vitro studies indicated that HR 756 was more potent in inhibiting *N gonorrhoeae* than other antibiotics, such as tetracyclines, cefamandole, and cefoxitin.[14] The present in-vivo study seems to confirm this because of the good clinical response to only 500 mg of the drug in a single dose. The dosage of cefuroxime used by other workers was 2 g.[9 10]

TABLE IV *Susceptibility of N gonorrhoeae to HR 756*

Strains	*Total*	*Minimum inhibitory concentration (μg/ml)* 0·03	0·016	0·008	0·004	0·002	0·001	0·0005	*Mean MIC*
PPNG	16	1	6	5	1	3			0·011
Non-PPNG	52		4	14	10	3	7	14	0·0045

TABLE V *Susceptibility of non-PPNG strains to HR 756 and penicillin G*

MIC of HR 756 (μg/ml)	*MIC of penicillin G (μg/ml)*						
	2	*1*	*0·5*	*0·25*	*0·125*	*0·062*	*0·031*
0·016	3	1					
0·008	3	4	7				
0·004			3	5	2		
0·002			2	0	1		
0·001				1	2	4	
0·0005						9	5

TABLE VI *Treatment failure according to susceptibility to HR 756*

		Failure	
MIC (μg/ml)	*No tested*	*No*	*%*
0·004	38	0	0
0·008	19	2	10·5
0·016	11	1	9·1

None of the three patients who failed to respond to treatment carried strains which were resistant to the antibiotic; the MICs were 0·016, 0·008 and 0·008 μg/ml. Correlation of the MICs with treatment response shows that failure may be expected with the present regimen when the MIC is 0·008 μg/ml or above. Further studies will be necessary to determine if this is so.

The overall tolerance to the drug was good. It is interesting to note that one of the patients who had a systemic reaction was originally hypersensitive to penicillin. This illustrates the possibility of cross-sensitisation between penicillin and cephalosporins. The drug did not affect the blood counts and biochemical tests in any of the patients. The dose may be too small for non-allergic toxic reactions to appear.

We conclude that the antibiotic, HR 756, is effective and safe, especially at the relatively low dosage of 500 mg. It is a good alternative to penicillin in the treatment of gonorrhoea because of its efficacy against the PPNG strains.

The authors thank Professor K Ė Chan and Mr William Ng of Hoechst (Singapore) Pte Ltd for the supply of drugs for use in this study.

References

1. World Health Organisation. *Neisseria gonorrhoeae* and gonococcal infections. *Tech Report Series 616* Geneva: WHO, 1978;91-104.
2. Malverson CW, Clarke EJ. Single-dose treatment of gonorrhoea with selected antibiotic agents. *JAMA* 1969;**210**:857-65.
3. Brownlow WJ, Watko LP, Aucoin EJ, Iglecia-Fernandes R. Treatment of gonorrhoea contracted in the Western Pacific region. *Br J Vener Dis* 1974;**50**:113-7.
4. Rajan VS, Tan NJ, Tan T, Khoo R, Sng EH, Pang CP. Treatment of gonorrhoea: the Singapore experience. *Asian J Infect Dis* 1977;**1**:71-4.
5. Fu KP, Neu HC. β-lactamase stability of HR 756, a novel cephalosporin, compared to that of cefuroxime and cefoxitin. *Antimicrob Agents Chemother* 1978;**14**:322-6.
6. Martin JE, Armstrong JH, Smith PB. A new system for cultivation of *Neisseria gonorrhoeae*. *Appl Microbiol* 1974;**27**:802-5.
7. Catlin BW. Iodometric detection of *Haemophilus influenzae* β-lactamase; rapid presumptive test for dampicillin resistance. *Antimicrob Agents Chemother* 1975;**7**:265-9.
8. Arya OP, Rees E, Percival A, Alergant CD, Annels EH, Turner GC. Epidemiology and treatment of gonorrhoea caused by penicillinase-producing strains in Liverpool. *Br J Vener Dis* 1978;**54**:28-33.
9. Fowler W, Rahim G, Brown JD. Clinical experience in the use of cefuroxime in gonorrhoea. *Br J Vener Dis* 1978;**54**:400-4.
10. Price JD, Fluker JL. The efficacy of cefuroxime for the treatment of acute gonorrhoea in men. *Br J Vener Dis* 1978;**54**:165-9.
11. Neu HC, Aswapokee N, Aswapokee P, Fu KP. HR 756, a new cephalosporin active against Gram-positive and Gram-negative aerobe and anaerobic bacteria. *Antimicrob Agents Chemother* 1979;**15**:273-81.
12. Wise R, Rollason T, Logan M, Andrews JM, Bedford KA. HR 756, a highly active cephalosporin: comparison with cefazolin and carbenicillin. *Antimicrob Agents Chemother* 1978;**14**:807-11.
13. Tan RJS, Sng EH, Rajan VS, Lim AL, Yeo KL, Lim EW. Evaluation of cefotaxime (HR 756)—a new cephalosporin against penicillinase-producing strains of *Neisseria gonorrhoeae*. *Asian J Infect Dis* 1978;**2**:239-41.
14. Murray PR, Christman JL, Medoff G. In-vitro activity of HR 756, a new cephalosporin against *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1979;**15**:452-4.

Br J Vener Dis 1980;56:259-62.

Contact-tracing in patients with genital chlamydial infection

I THELIN,* A-M WENNSTRÖM,* AND P-A MÅRDH†

*From the *Department of Dermatology and Venereology, University Hospital, and the †Department of Medical Microbiology, University of Lund, Lund, Sweden*

SUMMARY Of 2021 men attending a venereal disease clinic during a 20-month period, 527 (26·1%) harboured *Chlamydia trachomatis*, 310 (15·3%) *Neisseria gonorrhoeae*, and 94 (4·7%) both organisms. *C trachomatis* and *N gonorrhoeae* were isolated in 163 (15·7%) and 141 (13·8%) respectively of 1039 women attending the same clinic over a one-year period; 44 (4·2%) women harboured both organisms.

Contact-tracing was carried out for 112 male and 88 female patients infected with chlamydia, none of whom had gonorrhoea. Of the 144 female contacts, 103 (71·5%) attended for examination and 67 (65%) were found to harbour chlamydia. Of the 103 male contacts, 95 (92·2%) attended for examination and 50 (52·6%) were found to harbour chlamydia. Of the 67 female contacts, about 55% were symptomless as were 50% of the male contacts. Cultures for *N gonorrhoeae* from the sexual contacts of patients with genital chlamydial infections showed positive results in 37 (18·5%) of the 200 contacts examined.

For comparison the results of contact-tracing in 201 male and 231 female patients with gonorrhoea were analysed. Of the male and female contacts, 64·5% and 66·1% respectively attended the clinic for examination. *N gonorrhoeae* was isolated from 77·7% of the female and from 85·6% of the male contacts; about 40% and 50% respectively were symptomless.

The high percentage of symptomless carriers of *C trachomatis* among sexual contacts emphasises the need for tracing contacts of this infection.

Introduction

Chlamydia trachomatis is at least as common a cause of sexually transmitted infections as *Neisseria gonorrhoeae*.[1] Complications, such as salpingitis[2] and acute epididymitis,[3] seem to occur even more frequently in patients infected with chlamydia than in those with gonorrhoea.

As in all sexually transmitted diseases, it is important to treat not only patients infected with *C trachomatis* (including those who appear to be symptomfree) but also their sexual partners by tracing all sexual contacts to prevent further transmission of the organism. Such an epidemiological approach would be one way of reducing the incidence of infertility, which is a sequelae of salpingitis[4] and epididymitis. The importance of such measures should be considered in the light of recent reports that antibiotic treatment does not seem to reduce the incidence of infertility following salpingitis.[5]

Address for reprints: Dr P-A Mårdh, Department of Medical Microbiology, Sölvegatan 23, S-223 62 Lund, Sweden

Received for publication 14 October 1979

In Sweden contact-tracing of patients with gonorrhoea is required by law. Contacts named by the patient are informed that they may be carriers of *N gonorrhoeae*. They are requested to see a physician within a week of receiving the information. No such law exists regarding infections caused by *C trachomatis*, apart from lymphogranuloma venereum.[6]

In the present study, all patients attending a venereal disease clinic over a period of 12-20 months had cultures performed for *N gonorrhoeae* and *C trachomatis*. Apart from all patients with gonorrhoea, some of the patients in whom a chlamydial infection was diagnosed were asked if they would like to see a social worker to obtain further information about their infections; at this interview they were also told that their partners should be examined. The results of tracing the contacts of these latter patients are reported in this paper.

Patients and methods

STUDY POPULATION

The entire study population consisted of 2021 men and 1039 women, who attended the Department of

Dermatology and Venereology at the University Hospital, Lund, from September 1977 to April 1979 and from April 1978 to April 1979 respectively.

Most of the patients attending the department were between 15 and 25 years old, the median age of the women being slightly lower than that of the men. Almost all the patients were living either in the university town of Lund, which has about 50 000 inhabitants, or in the rural area surrounding the town. Many were students.

Contact-tracing of the partners of 112 of the men and of 88 of the women in whom genital chlamydial infections, but not gonorrhoea, had been diagnosed was undertaken.

CULTURAL STUDIES

C trachomatis

Urethral specimens for isolation of chlamydia were collected from the male patients and transported as described elsewhere.[2] In women, cervical specimens were collected with a cotton-tipped swab but were otherwise treated in the same way as the urethral specimens. Cultures for *C trachomatis* were made on cycloheximide-treated McCoy cells as previously reported.[8]

N gonorrhoeae

In addition to microscopical examination of urethral and cervical secretions, specimens were collected for culture for *N gonorrhoeae* from the urethra and rectum in men and from the cervix in women; these were inoculated directly on to a haematin agar medium,[9] which was immediately incubated at 37°C in an incubator with regulated CO_2 atmosphere and humidity. The methods used for the identification of *N gonorrhoeae* were those described elsewhere.[9]

CONTACT-TRACING

Gonorrhoea

All patients in whom gonorrhoea was diagnosed were seen by the social worker at the clinic to initiate tracing of contacts. In the present study, we analysed the results of contact-tracing in 231 of the women and 201 of the men in whom gonorrhoea had been diagnosed during the year preceding the present study. The prevalence of chlamydial infection in this group is not known. Either the patients were asked to contact their sexual partners themselves and to ask them to attend for examination or the social worker wrote a letter to the partners with instructions on what they had to do. According to Swedish law,[6] sexual contacts of patients with gonorrhoea who do not attend for examination can be compelled to do so; the initiative for this is then taken by the county medical officer after a report from the physician.

Chlamydial infection

Patients with genital chlamydial infections, apart from the very few patients with lymphogranuloma venereum, are not affected by a similar law. In their case, contact-tracing can only be undertaken on the basis of voluntary participation by the patient and their contacts. In this study, contact-tracing was carried out in 112 of the male and 88 of the female patients in whom a chlamydial infection, but not gonorrhoea, had been detected. In principle, contact-tracing was undertaken under the same conditions as for patients with gonorrhoea, although only after the patients and their contacts had given consent. Thus the patients with chlamydial infections were given the opportunity to talk to the social worker at the department, who asked the patients to contact their partners and suggest they attend for examination. The contacts were never asked directly by the social worker to attend the clinic. The patients were recommended to contact all those with whom they had had sexual contact during the three months or more before attending the clinic.

The patients in the two series were selected at random.

Results

TOTAL STUDY POPULATION

During the study period, 527 (26·1%) of the 2021 men were found to be infected with *C trachomatis. N gonorrhoeae* was isolated from 310 (15·3%) and both organisms were isolated from 94 (4·7%) male patients.

Of the 1039 women attending the clinic, 163 (15·7%) harboured *C trachomatis,* 141 (13·8%) *N gonorrhoeae,* and 44 (4·2%) both chlamydia and gonococci.

SYMPTOMS

Of the 527 men in whom a genital chlamydial infection was diagnosed, 134 (25·3%) considered themselves to be symptomfree. Similarly, 39 (12·6%) of 310 men with gonorrhoea and 16 (17%) men infected with both organisms had no symptoms.

Of the 163 women with chlamydial infections, 47 (28·8%) denied any symptoms as did 39 (27·7%) of the 141 women with gonorrhoea and 16 (36·4%) of the 44 women infected with both organisms.

CONTACT-TRACING

Chlamydial infection

The 112 men in whom contact-tracing for genital chlamydial infections was undertaken reported 144 female contacts. Of these women, 103 (71·5%) attended for examination and 67 (65%) were found to be infected with chlamydia; 55% of the 67 women denied any symptoms.

The 88 women with chlamydial infections in whom contact-tracing was performed reported 103 male contacts. Of these men, 95 (92·2%) attended for examination and 50 (52·6%) were found to harbour chlamydia; 50% denied any symptoms.

Gonorrhoea

Gonorrhoea was simultaneously diagnosed in 19 (18·4%) of 103 female and in 18 (18·9%) of 95 male contacts of patients with genital chlamydial infections.

The 201 men with gonorrhoea, in whom the results of contact-tracing were analysed, reported 242 female contacts. Of these women, 160 (66·1%) attended for examination and 137 (85·6%) were found to have gonorrhoea. The 231 women with gonorrhoea, in whom a similar analysis was made, reported 265 male contacts; of these, 171 (77·7%) were infected with gonorrhoea.

Of the 137 female contacts with gonorrhoea, approximately 40% were symptomfree, as were approximately 50% of the 171 male contacts infected with *N gonorrhoeae*.

Discussion

During recent years the importance of diagnosing and treating genital infections with *C trachomatis* has become obvious, as it is now known that chlamydia may cause not only primary genital infections but also serious complications. In our hospital catchment region, salpingitis is more often associated with chlamydial infection than with gonorrhoea.[10] In young men, chlamydia seem to be the most common cause of acute "idiopathic" epididymitis.[11] *C trachomatis* may also be transmitted to the neonates resulting in conjunctivitis and pneumonia.[1]

Contact-tracing has already been undertaken in selected groups of patients with genital chlamydial infections, such as in men with nongonococcal urethritis (NGU). In these studies, 30-70% of female contacts have been found to be chlamydia-positive.[12-15]

As indicated by the results of our contact-tracing, gonorrhoea seems to be more contagious than chlamydial infection. Thus gonococci were isolated from 81% and chlamydia from 59% of the contacts in the two series of patients studied. However, the less acute the symptoms were in patients with chlamydial infections the longer their infections lasted; furthermore, the presence of antibodies in secretions might make chlamydial infections more difficult to detect by culture than gonorrhoea.

In recent studies,[16] Paavonen found that in 25 of 99 chlamydia-positive female contacts of men with NGU, urethral but not cervical specimens gave positive results for *C trachomatis*. In comparative studies of such specimens from patients attending venereal disease clinics, we did not observe any difference in the isolation rate, using the same culture technique as in the present study (unpublished data).

The incubation period for genital infections with *C trachomatis* is generally longer than that for gonorrhoea and has been estimated to be 2-3 weeks.[1] This makes contact-tracing more difficult in patients infected with chlamydia than with gonorrhoea as they have to remember with whom they had sexual contact over a longer period. In our study the social worker noted the sexual history of the patients over a period of at least three months before their visit to the department. We found that many "three-month-old" contacts were carriers of *C trachomatis*.

Asymptomatic infections are more common in patients infected with *C trachomatis* than with *N gonorrhoeae*. Thus approximately half of the male and female chlamydia-positive contacts were symptomless. This makes contact-tracing important, as these patients are unlikely to seek medical advice.

When undertaking contact-tracing for patients with chlamydial infections, as for patients with gonorrhoea, the social worker should be informed of the range of clinical manifestations that might be caused by *C trachomatis*.

Despite the absence of a "lex veneris" for genital chlamydial infections (apart from lymphogranuloma venereum) similar to that for gonorrhoea, the patients generally realised the need for their sexual contacts to be examined. The percentage of contacts of patients with genital chlamydial infections who came for examination was higher than that for contacts of patients with gonorrhoea; the figure for the latter group, which attended the department, was low in comparison. This can be explained by the fact that they consulted other physicians.

The high percentage of symptomless carriers of chlamydia suggests that the organism may become widely spread in the community. The age group of persons in whom most genital chlamydial infections occur is however the same as that of those with gonococcal infections.

This study was supported by a grant (16X-4509) from the Swedish Medical Research Council.

References

1. Schachter J. Chlamydial infections. *N Engl J Med* 1978;**298**:428-35,490-5,540-9.
2. Mårdh P-A, Ripa T, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977;**296**:1377-9.

3. Berger RE, Alexander ER, Harnisch, JP, Paulsen, CA, Mondo GD, Ansell J, Holmes KK. Etiology, manifestations and therapy of acute epididymitis: Prospective studies of 50 cases. *J Urol* 1979; **121:**750-4.
4. Weström L. Effect of acute pelvic inflammatory disease on fertility. *Am J Obstet Gynecol* 1975; **121:**707-13.
5. Weström L, Iosif S, Svensson L, Mårdh P-A. Infertility after acute salpingitis: Results of treatment with different antibiotics. In: *Sexually Transmitted Diseases.* Reappraisal of the role of Vibramycin (doxycycline). New York: Science & Medicine Publishing Company Inc, 1979:47-50.
6. *Svensk Författningssamling,* No 231-235. Stockholm: Socialstyrelsen, 1968.
7. *Socialstyrelsens Författningssamling* No 93. Stockholm: Socialstyrelsen, 1977.
8. Ripa KT, Mårdh P-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol,* 1977; **6:**328-31.
9. Mårdh P-A, Mårtensson D, Soltesz LV. A new effective culture medium for *Neisseria gonorrhoeae. Sex Transm Dis* 1978; **5:**10-3.
10. Treharne JD, Ripa T, Märdh P-A, Weström L, Darougar S. Antibodies to *Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis,* 1979; **55:**26-9.
11. Berger RE, Alexander ER, Monda GD. *Chlamydia trachomatis* as a cause of "idiopathic" epididymitis. *N Engl J Med* 1972; **298:**301-4.
12. Oriel JD, Reeve P, Powis P, Miller A, Nicol CS. Chlamydial infection: Isolation of *Chlamydia* from patients with non-specific genital infection. *Br J Vener Dis* 1972; **48:**429-36.
13. Hilton AL, Richmond SJ, Molne JD, Hindley F, Clarke SKR. *Chlamydia* A in the female genital tract. *Br J Vener Dis* 1974; **50:**1-10.
14. Holmes KK, Hansfield HH, Wang S-P, *et al.* Etiology of nongonococcal urethritis. *N Engl J Med* 1975; **292:**1199-206.
15. Paavonen J, Kousa M, Saikku P, Vesterinen E, Jansson E, Lassus A. Examination of men with nongonococcal urethritis for *Chlamydia trachomatis* and *Ureaplasma urealyticum. Sex Transm Dis* 1978; **5:**93-6.
16. Paavonen J. Chlamydia-induced urethritis in female partners of men with nongonococcal urethritis. *Sex Transm Dis* 1979; **6:**69-71.

Br J Vener Dis 1980;56:263-6.

Confidentiality in the National Health Service and in the service for sexually transmitted diseases

R D CATTERALL
From the Department of Health and Social Security and James Pringle House, the Middlesex Hospital, London

SUMMARY The principles of medical confidentiality have been laid down by the Department of Health and Social Security to protect patients from unauthorised disclosure of confidential information, whether it is stored in traditional medical notes or in computers. Special arrangements have been made for the records of patients with sexually transmitted diseases since the inception of the service in 1916. In these cases medical secrecy is protected by statute law. Special regulations exist to allow disclosure of certain details to doctors, or persons employed under the direction of doctors, to facilitate contact tracing and treatment. Confidentiality is maintained in the specialty by the storage of records in the clinics, the refusal to allow information to be given outside the clinics except in special circumstances, the training of the clinic staff, and the absence of any central record of patients.

Introduction

The British Medical Association's handbook on medical ethics published in 1980[1] clearly sets out the doctor's obligation to maintain confidentiality when it states that, "it is the doctor's duty strictly to observe the rule of professional secrecy in refraining from disclosing voluntarily to any third party information which he has learned directly or indirectly in his professional relationship with a patient."

The World Medical Association's resolutions on medical secrecy and on computers in medicine[2] outline the values which underlie the concept of confidentiality by stating that, "the privacy of the individual is highly prized in most societies and widely accepted as a civil right." It continues; "the confidential nature of the patient-doctor relationship is regarded by most doctors as extremely important and is taken for granted by the patient." It warns that, "there is an increasing tendency towards an intrusion on medical secrecy." The 27th World Medical Assembly[3] reaffirmed; "the vital importance of maintaining medical secrecy, not as a privilege for the doctor but to protect the privacy of the individual, as the basis for the confidential relationship between the patient and his doctor; and it asked the United Nations, representing the people of the world, to give to the medical profession the needed help and to show ways for securing this fundamental right for the individual human being."

Hippocratic oath

Many patients have heard of the Hippocratic oath and believe that its sentiments have governed doctor-patient relationships since the fifth century BC. The BMA handbook on medical ethics,[1] for example, begins with the affirmation that the oath should form the foundation of medical ethics. The Royal College of Psychiatrists[4] asserts in a recent statement on ethics, "one of the few provisions of the Hippocratic oath which has remained unaltered over nearly 3000 years is that related to confidentiality." It quotes, "and whatsoever I shall see or hear in the course of my profession, as well as outside my profession in my intercourse with men, if it be what should not be published abroad, I will never divulge, holding such things to be holy secrets."

These sentiments are repeated in the Declaration of Geneva: "I will respect the secrets which are confided in me, even after the patient has died."[5]

There are, however, several difficulties about this popular concept of the 3000-year-old tradition of the Hippocratic oath. Firstly, it is probable that the vast majority of doctors qualify in medicine today without ever having read it or pondered on its implications. The lay notion that all doctors swear an Hippocratic oath on qualification is quite erroneous and the oath has never been a constant basis for medical practice throughout the ages.

Address for reprints: Dr R D Catterall, James Pringle House, the Middlesex Hospital, London W1N 8AA

Received for publication 7 December 1979

In fact, the oath originated in a small, esoteric cult on a minute island in the eastern Mediterranean and the obligations of secrecy were as much concerned with protecting trade secrets and maintaining control over initiates as they were with the patient's interests. It should be remembered that the oath only applied to the Hippocratic school of medicine and there were several other schools in antiquity without such requirements. When the mediaeval universities were established, with their faculties of medicine, new interest was shown in the oath by the developing guilds of physicians in an attempt to regulate healing practices, but its use did not become general. It was only in the late eighteenth and nineteenth centuries, when physicians and surgeons were attempting to achieve professional recognition and respectability, that the demand for an explicit code of professional practice became important. It was, therefore, the Victorian physicians and surgeons who adopted the oath and its concepts were widely diffused throughout the world.

One of the reasons for its apparent decline in importance during recent years is that modern medicine is not based on secrecy and rites of initiation but on widely shared scientific knowledge, on free publication, and on easy access to the results of medical research. These developments have resulted in new problems about the nature of confidentiality and they have been greatly enhanced by the anxieties expressed recently by the British Medical Association about the confidentiality of information on computers.[6]

Attitude of the DHSS

According to the Department of Health and Social Security (DHSS) all hospital records are the property of the Secretary of State for Health and Social Security. The attitude of the DHSS to confidentiality is clearly set out in the evidence given by the Department to the Data Protection Committee in November 1976:[7]

> (1) Patients should be protected from the unauthorised disclosure of confidential information compiled about them. Patients who supply personal information and submit to examination and treatment are entitled to expect that confidentiality will be fully protected.
>
> (2) Clinical records maintained by doctors (and other professional records) should be protected from unauthorised disclosure, including unathorised disclosure to a patient. This is essential if full and satisfactory records are to be maintained in the interest of the patient's well-being.
>
> (3) Patients will not co-operate fully in the compilation of information and doctors and other professional staff will be inhibited in maintaining suitable records, unless confidentiality is assured.

The confidentiality of information obtained from or compiled about National Health Service (NHS) patients should be safeguarded by the application of the following general rules:

> (1) Such information will not be used for any purposes other than those for which it was supplied or obtained without the consent of the doctor concerned, who will usually seek the consent of the patient. This applies both to personal details provided by the patient himself—such as name, address, age, occupation, and religion—and to information compiled by doctors and others relating to diagnosis, treatment, and care.
>
> (2) It is for the responsible doctor to decide through the exercise of his clinical judgment what information about his medical condition should be disclosed to his patient. Information about medical conditions and treatment contained in personal health records may not be disclosed to a patient by other members of the health care team, except with the consent of the doctor concerned. The patient does not have automatic right to access to information contained in his medical records, although the recent House of Lords judgment in the case of McIver and Another *vs* the Southern Health and Social Services Board,[8] concerning the disclosure of personal health records of patients engaged in legal proceedings, appears to provide one loophole in this general principle.
>
> (3) It is for the doctor concerned to decide initially whether confidential information about his patient should be disclosed voluntarily outside the health care team, whether to a relative of a patient or to any other person or agency. Medical ethics would usually require that the consent of the patient should be obtained to such disclosures, but the doctor concerned would decide whether or not, and in what circumstances, disclosure in a particular case, without the consent or even knowledge of the patient, was ethically acceptable. Disclosure to a third party without the patient's consent might, for example, be deemed appropriate where it was not in the interests of the medical well-being of a patient for him to learn of the nature of his illness. It would also arise in certain retrospective medical research studies where the doctor responsible for the records considered that it was impractical or clinically undesirable to seek the patient's consent.

Confidentiality is also safeguarded by the ethics of the medical profession and other bodies contributing to health care, as enforced by the professional bodies concerned. Doctors are answerable to the General

Medical Council and are expected to conform to the accepted ethics of the medical profession with respect to confidentiality.

The policy of the DHSS is that the same basic principles apply to all NHS patients' records, whether kept in manual systems or on computers, and that security arrangements should be tailored to the particular system of keeping patients' records to ensure that the basic principles of confidentiality are strictly applied and followed.

Service for sexually transmitted diseases

For over 60 years there have been special arrangements for ensuring the confidentiality of medical records of patients with venereal diseases in the United Kingdom. At the beginning of the century the Local Government Board had powers to ensure treatment of patients with infectious diseases under the Public Health Act of 1875 and 1896. In 1916, acting under these powers and on the recommendations of a Royal Commission, the Local Government Board issued regulations which required all local authorities to prepare schemes for approval by the Board for the treatment at public expense of all persons suffering from venereal diseases. The regulations also stressed that information about patients should be kept confidential. The venereal diseases were defined as syphilis, gonorrhoea, and soft chancre.

The United Kingdom service for venereal diseases evolved from these regulations and was provided in outpatient clinics attached either to hospitals or to the local authority public health facilities. No central direction was given on the way in which individual clinics should be organised and run and there was no guidance about methods of record-keeping. However, the number of cases of each of the three diseases treated at the centres had to be made available quarterly to the Chief Medical Officer. Because the quarterly returns dealt only with cases, the information was not identifiable in terms of individual patients. Drugs and medicines for the treatment of patients were to be free and provided at public expense. The scheme was, therefore, the first element in a free national health service and the first time that medical secrecy became statute law.

The Venereal Diseases Act 1917 made it a criminal offence for anyone other than a registered medical practitioner to treat for reward any person suffering from venereal diseases and an offence to advertise remedies for venereal diseases except in material sent to doctors and chemists.

During the second world war attempts were made to establish central control over the diagnosis and treatment of venereal diseases to try to prevent their spread from interfering with the war effort. In 1942, under Defence Regulation 33B, local authorities were given emergency powers to treat patients compulsorily and to notify details of them and their contacts to central government. The regulations were completely unsuccessful as doctors simply did not comply and many regarded them as an infringement of their duty of confidentiality to their patients and the regulations were repealed in 1947.

Only a very small number of cases were notified centrally, and the obvious failure of this attempt to introduce central control has been used as the basis for the argument that it would be counter-productive to attempt to impose any element of compulsion or notification into treatment. This has been the view taken by successive advisers to a number of post-war governments, and it is believed that the best guarantee that patients will attend clinics for diagnosis and treatment is the knowledge that all details will be absolutely confidential within the clinic.

National Health Service

The National Health Service Act 1946 provided a free health service for the people of the United Kingdom and the venereal diseases clinics, formerly run by local authorities, were incorporated into the new national hospital service. Certain regulations under this Act established the duty of confidentiality on regional hospital boards. But, owing to shortage of resources, some clinics continued to be run in existing buildings away from the main hospital complex and the contact tracers remained on the staff of the local authorities, which retained several public health functions. The increasing use of contact tracers, employed under the direction of clinicians to help check the spread of infection, did cause some problems, as several physicians held the view that the new regulations on confidentiality—which replaced those of 1916—prevented them from passing on details of patients to persons who were not employed by the National Health Service and were therefore not bound by its regulations.

As a result, replacement regulations were introduced in the National Health Service (Venereal Diseases) Regulations 1968 restressing the need for confidentiality but permitting disclosure of certain details about patients for the purpose of preventing the spread of disease. They are available in a memorandum issued by the DHSS in 1968[9] and make it quite clear that great importance is attached to the preservation of strict confidentiality of named information. The disclosure of information is permitted to a doctor, or a person employed under the direction of a doctor, to facilitate treatment and contact tracing. At the same time a memorandum on contact tracing in the control of venereal diseases[10] was sent by the Ministry of Health to all regional hospital boards, hospital management committees, and boards of governors. A copy of this document should be kept by all those concerned with contact tracing and particular attention should be given to the sections on confidentiality and on the legal aspects of contact tracing. Confidentiality is further stressed in the National Health Service (Venereal Diseases) Regulations 1974,[11] which state that steps should be taken to ensure that any information capable of identifying an individual shall not be disclosed except for the purpose of contact tracing and ensuring treatment.

The memorandum further states that hospital authorities should require both medical and other staff in venereal diseases clinics to keep strictly confidential any information they obtain about the identity of a patient and not to disclose it except as permitted under the regulations. This should be done either by including a requirement to this effect in their contract of service or by subsequent written instructions.

It is important to note that in the United Kingdom no information which would enable an individual to be identified is received centrally by the DHSS or any other organisations, as is the case in many other countries where registers of all those with positive serological test results for syphilis are kept centrally and, in some instances, individual detailed diagnoses are recorded. The exception to this is international contact tracing, when for convenience clinicians and communicable diseases centres channel information about contacts abroad of patients here and vice versa through the DHSS. This information is kept in strict medical confidence to a specially designated medical officer in the Department and is held only for transmission to the interested clinician.

Medical records of patients and details of contacts are held and stored in the clinics themselves and not in the main hospital record system. The DHSS is not aware of any identifiable record of patients with sexually transmitted diseases being held on a computer. Clinicians and contact tracers exchange information about contacts without central intervention and the physicians in charge of clinics are responsible for returning statistics of cases treated, analysed by age group and sex, quarterly to the Chief Medical Officer to the DHSS. These figures, which report cases only, are published in the annual report of the Chief Medical Officer of Health and are subsequently printed in the *British Journal of Venereal Diseases.*

Confidentiality is further safeguarded by the fact that referral to the clinics is usually on the patient's initiative and does not require an introductory letter of reference from the patient's general practitioner, as is the case with other non-emergency hospital visits. In these cases the clinic physician does not normally inform the patient's general practitioner unless there is some special reason for doing so. However, an increasing number of patients are being referred to clinics by their family doctors and by consultants in other fields and in these cases a full report is sent to the referring physician with an indication of the confidential nature of the communication.

Conclusions

In general, the principal aim of the STD service is to eliminate, as far as possible, any barriers between the patient and his or her access to specialist diagnosis and treatment. Confidentiality is assured, as far as possible, by the conduct of the clinic staff, the decentralisation of records, the ban on identifiable information leaving the clinic except in special circumstances, and the absence of a central record of patients.

References

1. British Medical Association. *The Handbook of Medical Ethics.* London: BMA, 1980.
2. World Medical Association. Medical secrecy and computers in medicine. *World Med J* 1979;26:4-6.
3. 27th World Medical Assembly. Resolutions on medical secrecy. *World Med J* 1973;20:120.
4. Special Committee on Confidentiality and Medical Information Systems. *Bull R Coll Psychiatrists* 1979:103-5.
5. 22nd World Medical Assembly. The Declaration of Geneva. *World Med J* 1968;14:16-24.
6. British Medical Association. Confidentiality, records, and computers. *Br Med J* 1979;1:698-9.
7. Department of Health and Social Security. *Report of the Committee on Data Processing*. London: HMSO, 1978.
8. McIvor *vs* the Southern Health and Social Services Board, *WLR* 757, 1978.
9. Department of Health and Social Security. *Control of Venereal Diseases.* HM (68) 84. London: DHSS, 1968.
10. Department of Health and Social Security. *Memorandum on Contact Tracing in the Control of Venereal Disease.* HM(68)84. London: DHSS, 1968.
11. Department of Health and Social Security. *The National Health Service (Venereal Diseases) Regulations 1974.* London: DHSS, 1974.

Br J Vener Dis 1980; **56**: 267-8.

Condylomata lata of the palms: an unusual site

M EL-SAAD EL-RIFAIE
From the Department of Dermatology, Kasr El-Eini Faculty of Medicine, Cairo University, Cairo, Egypt

SUMMARY In an unusual case of condylomata lata of the palms the development of moist hypertrophic papular eruptions is probably explained by the fact that the patient was a car-driver and had hyperhidrosis of the palms.

Introduction

Cutaneous lesions of secondary syphilis are very variable and simulate a wide variety of skin diseases. Condylomata lata, or syphilitic moist papules, appear usually in moist and warm areas of the body, particularly the anogenital region. In this report a case is described of condylomata lata on the palms of a car-driver with palmar hyperhidrosis.

Case report

A Yemenese heterosexual 25-year-old man was referred to the clinic of skin diseases in the King Faisal Hospital, Taif, Saudi Arabia, with a tongue lesion. The patient had consulted the doctor because of a low-grade fever, malaise, headache, and hoarseness of the voice of one week's duration; he was unaware of the palmar and tongue lesions.

On examination, the patient had generalised lymphadenopathy. The skin was clinically clear except for hyperhidrosis of the palms and numerous, dull greyish-white, moist papules (6-10 mm in diameter), which occurred in particular on the creases of the right hand opposite the metacarpophalangeal joints (figure). The papules were flat, eroded, and oozing. The soles of the feet were not affected. Greyish-white mucus patches were seen on the tongue, although other mucus membranes appeared healthy.

Darkground examination of the moist papules and mucous patches showed *Treponema pallidum*. The Venereal Disease Research Laboratory (VDRL) and fluorescent treponemal antibody absorption (FTA-ABS) tests gave positive results. The patient stated that he had frequent sexual relations with prostitutes and had had an infected ulcer on the penis for two months before the present illness. He had treated the penile lesion himself with some local medication.

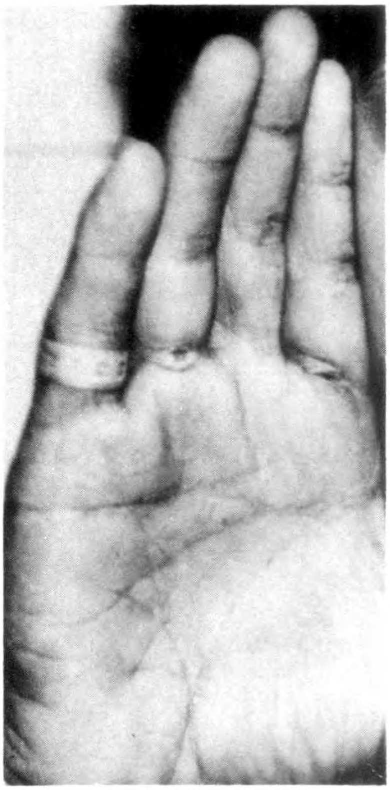


FIG *Condylomata lata of the palms*

The palmar lesions gradually resolved after treatment with two intramuscular injections of benzathine penicillin 2·4 megaunits at a week's interval.

Discussion

Condylomata lata are usually seen in moist and warm areas of the body, such as the anogenital region. Solitary lesions were reported in the toe-webs by Minkin *et al*,[1] and extensive condylomata lata were

Address for reprints: Dr M El-Saad El-Rifaie, Department of Dermatology, Cairo University, 79 Kasr El-Eini Street, Cairo, Egypt

Received for publication 22 October 1979

reported in an Indian patient by Shrivastava and Singh.[2] In the present case, the site of the condylomata is unusual and, as far as I know, has not been reported before. Holding the steering wheel for long periods during driving together with hyperhidrosis of the palms probably explains the unusual site of this eruption. Cutaneous lesions of secondary syphilis may be inconspicuous, particularly when they are in unusual sites. In such cases a thorough clinical examination is important.

The author is indebted to Dr G El-Guindy for his co-operation.

References

1. Minkin W, Landy SE, Cohen HJ. An unusual solitary lesion of secondary syphilis. *Arch Dermatol* 1967;95:217.
2. Shrivastava SN, Singh G. Extensive condylomata lata. *Br J Vener Dis* 1977;53:23-5.

Br J Vener Dis 1980; **56**: 269-70.

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Effect of pH on the motility and virulence of *Treponema pallidum* (Nichols) and *Treponema paraluis-cuniculi* in vitro under anaerobic conditions

Sir,

No published studies have reported the effect of pH on the retention of virulence of *Treponema pallidum*, as measured by rabbit inoculation, as distinct from motility retention in vitro. Using the latter technique, an optimum pH of 7·2 was reported.[1] Furthermore, to the best of our knowledge no in-vitro studies of any kind have been performed with *Treponema paraluis-cuniculi*, the causative agent of rabbit venereal spirochaetoses, and its pH optimum is not known.

We have demonstrated that for both organisms the optimum pH for retention of motility was 7·3-7·6 (figure). However, the more sensitive parameter of virulence retention showed that pH 7·3-7·4 was optimal for both *T pallidum* and *T paraluis-cuniculi* (table). Both treponemes were more resistant to alkaline than acidic changes from their optimum pH.

The medium used in our experiments was a slight modification of an anaerobic medium with low redox potential.[2] The experimental pH variants were obtained by adding a few drops of 0·1 mol/l NaOH or 0·1 mol/l HCl to the final medium. Very little was required as the medium was poorly buffered with phosphate. Freshly harvested *T pallidum* (or *T paraluis cuniculi*) obtained from the orchitic testes of rabbits inoculated with *T pallidum* two weeks previously (or *T paraluis cuniculi* four weeks previously) were at a concentration of approximately 10^7 treponemes/ml, and 0·5-ml volumes of these preparations were added to 10 ml of medium under anaerobic conditions. The pH and redox potentials were measured daily to ensure that no changes had occurred. The pH variant media were all isotonic for sheep erythrocytes. Incubation temperature was 34°C under anaerobic conditions. Determinations of treponemal motility and virulence were performed at 24-hour intervals, the former by darkfield microscopical examination of 200 treponemes from duplicate culture tubes and the latter by the quadruplicate inoculation of 0·1 ml of culture intradermally into the shaved backs of rabbits. The rabbits were kept shaved, cool (18°C), and examined daily for the development of syphilitic lesions until no further lesions appeared.

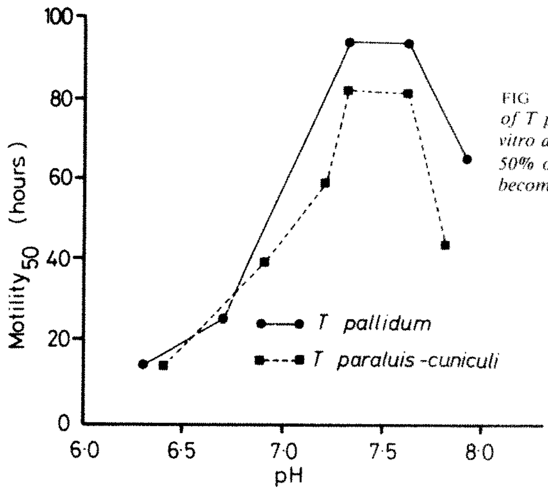


FIG *Effect of pH on motility retention of T pallidum and T paraluis-cuniculi in vitro as measured by the time taken for 50% of the treponemes in culture to become immotile (motility$_{50}$).*

TABLE *Effect of pH on survival of virulent T pallidum and T paraluis-cuniculi in vitro as measured by latent period of infection in the rabbit*

Incubation period (days)	*Latent period* of infection (days) after in-vitro incubation at defined pH*					
T pallidum	*6·3*	*6·7*	*7·3*	*7·6*	*7·9*	
0	7	7	7	7	7	
1	†	13	7	7	7	
2	†	20	11	12	12	
3	†	†	12	12	15	
4	†	†	12	18 (2/4‡)	†	
T paraluis-cuniculi	*6·4*	*6·9*	*7·2*	*7·4*	*7·6*	*7·8*
0	14	12	12	14	15	15
1	†	18	17	18	18	18
2	†	†	22 (1/4‡)	19	27	35 (1/4)†
3	†	†	†	30 (2/4‡)	†	†

* Period (in days) between rabbit inoculation and appearance of syphilitic lesions as indicated by induration of the skin.
† No lesions developed from four inoculation sites on rabbit.
‡ No of inoculation sites that developed into syphilitic lesions compared with total No of inoculation sites. Where no figures in parentheses are given the value is 4/4.

In view of our findings, we recommend that further studies on the growth in vitro of virulent *T pallidum* and *T paraluis-cuniculi* should be carried out at pH 7·3-7·4.

We wish to thank Mr Ian McLean for his excellent technical assistance. The *T paraluis-cuniculi* (strain 8816) was kindly supplied by Dr A Balows (Centre for Disease Control, Atlanta).

The work was supported by the National Health and Medical Research Council (Australia), the Utah Foundation, the Potter Foundation, the Danks Trust, the estate of the late George Adams and Monash University, from whom financial assistance is gratefully acknowledged.

Yours faithfully,
S Graves
T Goty
F Trewartha

Department of Microbiology,
Monash University,
Victoria, Australia

References

1. Metzger M, Smogor W. Study of the effect of pH and Eh values of the Nelsen-Diesendruck medium on the survival of virulent *Treponema pallidum*. *Arch Immunol Therap Exp* 1966; **14**: 445-53.
2. Graves S, Sandok P, Jenkin H, Johnson R. Retention of motility and virulence of *Treponema pallidum* (Nichols strain) in vitro. *Infect Immun* 1975; **12**: 1116-20.

TO THE EDITOR, *British Journal of Venereal Diseases*

β-lactamase-producing gonococci in Hong Kong

Sir,

From 19 October 1979 to 26 December 1979 15 cases of gonorrhoea were confirmed by culture. Of these 15 cases, three were due to β-lactamase-producing gonococci. All 15 cases were Chinese patients encountered in private practice.

Specimens for culture were taken with a sterile cottonwool bud from the urethra in men and from the cervix in women and inoculated on to Thayer-Martin media. These specimens were then transferred to the Department of Extramural Studies of the University of Hong Kong for incubation. The identity of suspected colonies of *Neisseria gonorrhoeae* was confirmed by the oxidase test, Gram-stained smear, and sugar fermentation. β-lactamase-producing gonococci were detected by the chromogenic cephalosporin test.

A male patient, aged 32 years, presented with dysuria and a purulent urethral discharge. The source of infection was a prostitute in Hong Kong. He was successfully treated with spectinomycin 2 g intramuscularly.

A female patient, aged 22 years, presented with dysuria and a purulent vaginal discharge. She was a contact of the previous patient and was successfully treated with spectinomycin 2 g intramuscularly.

A male patient, aged 23 years, presented with dysuria and a purulent urethral discharge. The source of infection was a prostitute in Hong Kong. He was treated with kanamycin 2 g intramuscularly. Unfortunately the patient did not attend for follow up.

Yours faithfully,
F H Wang

Office A, 1/F Fuji Building,
381-383 Lockhart Road,
Hong Kong

Notices

Project Icarus
Project Icarus is an independent registered charity producing visual aids for health education. Most of these visual aids are posters and they include a set of eight posters on venereal disease (size 16 in × 22 in). Other sets of posters cover drug abuse, alcoholism, and family planning. Each poster is black and white and is available in single editions or in sets (£2·30 per set or 40p each including carriage). Further information can be obtained from: Project Icarus Ltd, Raglan House, 4 Clarence Parade, Southsea, Hants PO5 3NU; Tel: Portsmouth (0705) 27460.

Atlas of sexually transmitted diseases
An "Atlas of Sexually Transmitted Diseases" by Professor E Stolz and Dr J van der Stek, published by Boehringer Ingelheim, is available from the UK company—namely, Boehringer Ingelheim, Southern Industrial Estate, Bracknell, Berks RG12 4YF. Doctors interested in obtaining copies should write direct to the UK company at this address.

Correction

In the paper by Evans *et al* on the "Prolonged use of the Greenland method of treatment of gonorrhoea" (1980; **56**: 88-91) the minimum inhibitory concentration quoted in the first sentence of the introduction should have read ⩾0·1 μg/ml and not ⩽0·1 μg/ml.

Br J Vener Dis 1980;56:271.

Book reviews

Venereology and Genitourinary Medicine. By R D Catterall, 1979. 2nd ed. Hodder and Stoughton, London. Price £3·25.

The second edition of this popular book has been rewritten to include several recent advances in the subject. It provides a comprehensive guide to the sexually transmitted diseases, their epidemiology, management, and treatment, with chapters on contact tracing and the role of the nurse, both of which are invaluable. Other non-sexually transmitted conditions affecting the genitalia are also discussed as well as the many psychological and psychosexual problems.

It has a very useful glossary of terms and a good index. It also contains several black-and-white line drawings and graphs. In the treatment of syphilis there is a very good section on the penicillins and the diagnosis and management of reactions to them.

It is a pity that the book is spoilt by many very obvious misprints; for example syphilis is said to have first appeared in England in 1947 instead of 1497 and the word "sphygmomanometer" is spelt as "sphygmotonometer" and "heels" is spelt "heals". There are many other misprints, and it would benefit the publishers to have the proofs thoroughly reread before the next printing. However, this book is excellent for all grades of nurses, especially those working in clinics for sexually transmitted diseases, and is a sound introduction to the subject for medical students and, indeed, doctors.

At £3·25 it is very reasonably priced and is handy to carry around in the pocket. It should be an essential addition to all nursing and medical libraries.

P K Taylor

Human Reproduction. By R W Huff and C J Pauerstein, 1979. John Wiley and Sons Ltd, New York, USA. Price £9.

This 500-page, concise and well-written book is based on the content of lectures given to undergraduates at the University of Texas Medical School and encompasses the reproductive sciences in three sections: reproductive biology, gynaecological pathophysiology, and pregnancy.

The overall content of the volume is excellent with well-judged detail in most parts, although it was disappointing to see no mention of chlamydia or tuberculosis in the discussion of genital tract infections, whereas other infections are dealt with at length.

Illustrations might have been used more liberally at the expense of many space-wasting rambles such as, "pregnancy . . . represents a new disequilibrium state requiring a solution through maturational reorganisation . . ." Indeed, where photomicrographs do occur they are of poor quality and detract from the text.

The 24 chapters are divided into readable sections by logical subheadings, which make the book easy to use although it may be that both undergraduates and postgraduates will be confused by the differences in definitions of such events as abortion and maternal mortality used in this book and those used in the UK. In general, the modern style of the book will please most undergraduates, who will appreciate the list of objectives at the beginning and the multiple-choice questions at the end of each chapter.

Simon G Crocker

The Love Diseases. By Paul Redfern, 1979. MacMillan Press (Papermac), London. Price £2·50.

This book is a layperson's guide to promiscuity spiced with language and comments intended to stimulate the reader and promote the sales. The author is a medically qualified freelance writer who tells us that he has "great scope and stamina for sexual intercourse." This experience enables him to dispense advice, such as "the trick is to keep yourself in shape sexually with masturbation" and "much better to screw around than become a withdrawn wet." The book is laid out under a list of entries in alphabetical order from "abortion" to "where to look." Several line drawings and numerous quotations appear on the left hand pages, including John Donne, Boswell, Mae West, Phillip *(Portnoy's Complaint)* Roth, and some distinguished colleagues in the specialty. From a medical standpoint the text is substantially accurate and commendably up to date, thanks to Dr Anthony Wisdom, who contributes a foreword, but a number of important errors have appeared.

There is reference to cystitis as a complication of gonorrhoea in the male (p 62), which must be extremely rare, but no mention that it can be symptomless—of vital importance in a highly promiscuous male. To state that, "the alcohol restriction is unnecessary" as part of the management (p 64) is at the very least unhelpful, bearing in mind the increasing prevalence of postgonococcal urethritis and the depressing problem of relapsing non-specific urethritis. Hepatitis B infection (especially in its chronic form) may destroy the liver, but the reader is told only that it "may be . . . so mild that the patient isn't ill enough to go to the doctor," which is irresponsible (p 69). Moreover, swallowing semen is not the common factor in its spread. Cytarabine is a powerful cytotoxic, certainly not used for superficial herpetic infection, and the recommendation to get an injection at the onset of symptoms is ludicrous (p 71). Advice to visit the clinic before further sexual contact with one's wife, husband, or friend if "you've had one of the locals while away" (p 106) is more likely to engender a false sense of security, since diagnosis of incubating infection is not possible. In other words, there really is no substitute for a period of sexual abstinence to ensure that nothing will be passed on, however unpalatable that may be, and attending a clinic will not alter the fact.

The Love Diseases was written for people in their late teens to help them cope better with the vicissitudes of love and sex, but in some respects it may well compound their problems, not least by transmitting the author's obsession with sexual excess.

B A Evans

Br J Vener Dis 1980;**56**:272-5.

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea
(Clinical; microbiology; therapy)
Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases
Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (clinical and therapy)

Acquired syphilitic uveitis
WH ROSS AND HFS SUTTON (St Paul's Hospital, Vancouver, Canada). *Arch Ophthalmol* 1980;**98**:496-8.

Secondary syphilis is an overlooked cause of iritis and posterior uveitis. Three cases of acquired syphilitic uveitis were observed, two of which occurred in male homosexuals. In the first three patients, a delay in diagnosis and treatment led to irreversible loss of vision and visual field. In the latter two cases, an increased index of suspicion led to earlier diagnosis and treatment with complete recovery of vision.

Authors' summary

Congenital neurosyphilis and juvenile paresis. A forgotten entity?
J WIGGELINKHUIZEN AND R MASON (University of Cape Town, South Africa). *Clin Pediatr* 1980;**19**:142-5.

Syphilis (serology and biological false-positive phenomenon)

Serologic responses to treatment of 128 patients with late latent syphilis
NJ FIUMARA (Boston University School of Medicine, Boston, USA). *Sex Transm Dis* 1979;**6**:243-6.

One hundred and twenty-eight patients who had been treated for late latent syphilis were followed. Benzathine penicillin G ($2\cdot4\times10^6$ units given intramuscularly once a week for two weeks) was given to 123 patients, and five patients were given tetracyclines (500 mg orally four times daily for 12 days). Of these patients, 56 (44%) became seronegative within five years and 72 (56%) had persistently positive reagin test results.

Author's summary

Syphilis (pathology and experimental)

Molecular characterization of receptor binding proteins and immunogens of virulent *Treponema pallidum*
JB BASEMAN AND EC HAYES (University of North Carolina, USA). *J Exp Med* 1980;**151**:573-86.

Gonorrhoea (clinical)

Aseptic arthritis after gonorrhoea
L ROSENTHAL, B OLHAGEN, AND S EK (Karolinska Sjukhuset, Stockholm, Sweden). *Ann Rheum Dis* 1980;**39**:141-6.

Sixteen patients with aseptic arthritis developing after gonorrhoea and 14 patients with arthritis after nongonococcal urogenital infection have been analysed with respect to clinical course, roentgenological signs, and humoral as well as cellular immune responses to *Neisseria gonorrhoeae* antigen. Fifty-eight healthy blood donors were used as controls. The clinical pattern did not differ significantly between the two groups. Eye or skin lesions indicative of Reiter's syndrome were found in five patients of both groups. Signs of sacroiliac arthritis were found in eight and six patients respectively. Gonococcal complement fixation gave a positive result in nine of 16 patients in the postgonococcal arthritis group and in none of 14 patients in the arthritis group with nongonococcal urogenital infection. The lymphocyte stimulation induced by gonococcal antigen was significantly greater in patients with postgonococcal arthritis than in healthy controls. When reference was made to the results of stimulation of the lymphocytes with PPD, there was also a significant difference in the lymphocyte reactivity to gonococcal antigen between the group of patients with postgonococcal arthritis and that of patients with arthritis after nongonococcal urogenital infection. No such difference was noted between the latter group and the healthy controls. The clinical and immunological data support the hypothesis that *Neisseria gonorrhoeae* may induce an aseptic arthritis which sometimes presents as a complete Reiter's syndrome.

Authors' summary

Colonization of the pharynx with *Neisseria gonorrhoeae*: experience in a clinic for sexually transmitted diseases
WA OSBORNE AND L GRUBIN (University of Connecticut, USA). *Sex Transm Dis* 1979;**6**:253-6.

Material from the pharynx of 1453 patients (841 men and 612 women) who attended a sexually transmitted diseases clinic was cultured for *Neisseria gonorrhoeae*. The organism was isolated from the throat of 19 (2%) men and 23 (4%) women. Infection was confined to this site in eight men and 13 women. Eleven heterosexual men became infected during cunnilingus; the other men with pharyngeal gonorrhoea had had homosexual contact.

A McMillan

Epidemiologic follow-up study of patients with gonococcal pelvic inflammatory disease

LB VOLKIN, DS THOMPSON, AND GG SHEPERD (University of Pittsburgh, Pennsylvania, USA). *Sex Transm Dis* 1979;**6:**267-9.

Gonorrhoea (microbiology)

Association between auxogroup of *Neisseria gonorrhoeae* and the minimal inhibitory concentration of penicillin

IO STEWART AND AT HENDRY (McMaster University, Ontario, Canada). *Sex Transm Dis* 1979;**6:**247-52.

Detection of 3-hydroxy fatty acids at picogram levels in biologic specimens: chemical method for the detection of *Neisseria gonorrhoeae*

IJ SUD AND DS FEINGOLD (Veterans Administration Hospital, Boston, USA). *J Invest Dermatol* 1979;**73:**521-6.

Identification of organisms by Gas liquid chromatography (GLC) usually depends on the recognition of specific patterns of microbial metabolites. This study reports a method to identify a bacterial component, 3-hydroxy dodecanoic acid (3-OH C12:0 or β-hydroxylauric acid), as a marker for the presence of *Neisseria gonorrhoeae*. Neisseria differ from most other Gram-negative bacteria in that 3-OH C12:0 is the major hydroxy acid and is responsible for 10-20% of the total fatty acid content of the cell; in most Gram-negative bacteria, 3-hydroxy tetradecanoic acid (3-OH C14:0 or β-hydroxymyoistic acid) is the major hydroxy acid and 3-OH C12:0 is less than 5% of the quantity of 3-OH C14:0. The method reported is complicated; it involves several time-consuming procedures, including the preparation of a heptafluorobutryl derivative of the butyl ester of the fatty acid and its purification and detection by GLC using an electron capture detector.

Thirty-one cervical specimens with known culture results for *N gonorrhoeae* were examined by the GLC method. Of 19 culture-positive specimens, 15 were found to be positive for the presence of gonococci by detecting 3-OH C12:0. Of the 12 culture-negative specimens, three were identified as positive by GLC. Organisms that are frequently found in vaginal flora (*Lactobacillus plantanium, Peptostreptococcus anaerobius, Peptococcus* species, and *Bacteroides fragilis*) were examined and found to be devoid of 3-OH C12:0. Limitations of the method are that about 10^5 gonococci are required for detection and that other neisseriae if present in the genital tract in these numbers would also give a positive result. The method requires further study, particularly with a view to simplifying the methodology and decreasing the time required for processing specimens.

H Young

Detection of antibodies reactive with *Neisseria gonorrhoeae* in secretions on extra-genital surfaces

A McMILLAN, H YOUNG, AND G McNEILLAGE (University of Edinburgh, UK). *J Infect* 1980;**2:**53-9.

Using an immunofluorescent antibody test, antigonococcal antibody was found in the rectal secretions of six of 18 men with rectal gonorrhoea; this was of the IgG class in all six and associated with IgA in three. This antibody was only detected in two of 14 women with rectal infection.

Antibody reactive with *Neisseria gonorrhoeae* was found in the saliva of each of 12 men and in four of six women with oropharyngeal gonorrhoea.

Salivary antibody was found in 10 of 18 homosexual, and in two of 25 heterosexual, men with urethral or rectal gonorrhoea but without apparent pharyngeal infection ($p<0\cdot05$).

Authors' summary

Serology of *Neisseria gonorrhoeae*. Classification by co-agglutination

E SANDSTRÖM AND D DANIELSSON (Sodersjukhuset, Stockholm, Sweden). *Acta Pathol Microbiol Scand (B)* 1980; **88:**27-38.

The co-agglutination (COA) method has been adapted for serological classification of *Neisseria gonorrhoeae*. COA reagents were prepared with selectively absorbed rabbit hyperimmune antibodies against gonococcal (GC) major outer membrane protein (MOMP) serotype strains. Using these reagents, the 16 MOMP reference strains could be referred to at least three antigen classes, tentatively named W, J, and M. The GC antigens of class W were divided into three groups—I, II and III—and they were in part sensitive to pronase. The antigens of class J reflected strain-specific or serotype reactions, some sensitive and others resistant to proteolytic enzymes. The antigens of class M were sensitive to periodate and resistant to pronase. Strains used in serological studies by other authors were tested. The properties of class W correlated well with those of the so-called micro-immunofluorescence and immunotype systems, and class M with those of the so-called endotoxin and acid polysaccharide systems. Strains from three different laboratories could all be grouped by class W and M reagents, identical strains obtained independently from different laboratories gave very similar reaction patterns with the reagents available. Repeated gonococcal isolates from patients infected with β-lactamase-producing strains showed stable reactions with class W and J reagents while there was a time-related variation of the class M pattern. We have found that the COA method is rapid, easy, and reproducible in the serological classification of *Neisseria gonorrhoeae*, and all the 117 gonococcal strains tested could be classified.

Authors' summary

Serology of *Neisseria gonorrhoeae*. Demonstration by co-agglutination and immunoelectrophoresis of antigenic differences associated with colour/opacity colonial variants

D DANIELSSON AND E SANDSTRÖM (Central County Hospital, Orebro, Sweden). *Acta Pathol Microbiol Scand (B)* 1980;**88:** 39-46.

Characterization of rabbit hyperimmune antisera by line-rocket immunoelectrophoresis for use in co-agglutination

E SANDSTRÖM AND D DANIELSSON (Sodersjukhuset, Stockholm, Sweden). *Acta Pathol Microbiol (B)* 1980;**88:**17-26.

Purine metabolism in *Neisseria gonorrhoeae:* the requirement for hypoxanthine

SA MORSE AND L BARTENSTEIN (University of Oregon, USA). *Can J Microbiol* 1980;**26:**13-20.

Growth of *Neisseria gonorrhoeae* in continuous culture

RJ MANCHEE, CN WIBLIN AND A ROBINSON (Chemical Defence Establishment, Salisbury, UK). *FEMS Microbiol Lett* 1980;**7:**115-8.

Stabilization and purification of ornithine transcarbamylase from *Neisseria gonorrhoeae*

CN POWERS AND DL PIERSON (Baylor University, Texas, USA). *J Bacteriol* 1980; **141:**544-9.

Gonorrhoea (therapy)

Pharyngeal infection with *Neisseria gonorrhoeae*

NJ FIUMARA (Boston University School of Medicine, Boston, USA). *Sex Transm Dis* 1979; **6:**264-6.

During 1978, 156 patients were treated for pharyngeal infection with *Neisseria gonorrhoeae;* 115 (74%) returned for a post-treatment culture. Treatment with aqueous procaine penicillin G ($4\cdot8\times10^6$ units) given intramuscularly with (19 patients) or without (64 patients) orally administered ampicillin for four days was successful in 86% of cases. All seven patients who received tetracycline hydrochloride orally for five days had negative results to post-treatment cultures as did nine of 12 patients who had received 2·0 g (four patients) or 4·0 g (eight patients) of spectinomycin hydrochloride intramuscularly. Concurrent anogenital infection with *Neisseria gonorrhoeae* was documented for about 60% of the patients and, with one exception, was eradicated by these treatment schedules.

Author's summary

Cefoxitin in the treatment of gonorrhoea

RB JONES, J STIMSON, GW COUNTS, AND KK HOLMES (University of Washington, Seattle, USA). *Sex Transm Dis* 1979; **6:**239-42.

Rectal gonorrhea in men: diagnosis and treatment

DA LEBEDEFF AND EB HOCHMAN (Los Angeles, USA). *Ann Intern Med* 1980; **92:**463-6.

In a prospective investigation of rectal gonorrhoea in men, 1262 patients were studied on the basis of rectal symptoms, contact with gonorrhoea, or a previously positive rectal culture result for *Neisseria gonorrhoeae.* Of these, 554 (44%) patients had rectal gonorrhoea based on a positive culture result; in these patients, mucus in the stool, together with the anoscopic finding of generalised exudate, proved relevant but unhelpful when compared with symptoms and findings in culture-negative patients. Patients were treated with either procaine penicillin 4·8 million units with probenecid 1 g, ampicillin 3·5 or 4·5 g with probenecid 1 g, or tetracycline 9·5 g given over four days. Four hundred and seven patients with rectal gonorrhoea returned for test-of-cure examination. Failure rates were 5% with each of the first three regimens and 15% with tetracycline ($P<0\cdot01$). Procaine penicillin with probenecid is recommended as the treatment of choice, with tetracycline being reserved for penicillin-allergic patients.

Authors' summary

Treatment of anorectal gonorrhea infections in men

M SANDS (San Francisco City Clinic, USA). *JAMA* 1980; **243:**1143-4.

Non-specific genital infection

Chlamydial pneumonia of infancy: further clinical observations

VH SAN JOAQUIN, JR HERRIN, AND JM HAUTALA (University of Oklahoma, USA). *Clin Pediatr* 1980; **19:**109-12.

Two infants with pneumonia attributed to infection with *Chlamydia trachomatis* presented at eight and six weeks with difficulty in breathing associated with vomiting and nasal congestion. Both were noted to have conjunctivitis dating from shortly after birth.

On examination the 8-week-old girl was tachypnoeic, and bilateral wheezes with rales were noted. Relevant laboratory results were negative except for a serum immunofluorescent antichlamydial antibody titre of 1/65 536. Chest radiographs showed hyperinflation with bilateral extensive infiltration. The baby improved and remained asymptomatic after a seven-day course of ampicillin and gentamycin.

The boy was admitted at 6 weeks old after a course of sulphacetamide eyedrops as he had developed tachypnoea with expiratory rales and ronchi. Chest radiographs showed bilateral hyperaeration with streaky infiltrates of the left upper lobe. Epinephrine cleared the wheezes initially but congestion, cough, and mild tachypnoea were noted at 5 months old when he presented with bilateral otitis media. Laboratory investigations were unremarkable except for serum immunofluorescent antichlamydial antibody titre in excess of 1/65 536 with a concomitant maternal titre of 1/1024. A prolonged course of sulfisoxazole eventually ensured complete resolution. Unfortunately culture for *C trachomatis* is not reported for either case.

In conclusion it is stressed that chlamydial pneumonia of infancy should be considered in cases of possible bronchiolitis or bronchial asthma in infancy and that wheezing may be a predominant clinical manifestation. Spontaneous resolution may occur, as in the first case, where antibiotics unacceptable in treating chlamydial infections were used; alternatively, the infection may become persistent and protracted, as in the second case, where final treatment was given when the child was 8½ months old.

R S Pattman

Perihepatitis and chlamydial salpingitis

P WØLNER-HANSSEN, L WESTRÖM, AND P-A MÅRDH (University of Lund, Sweden). *Lancet* 1980; **i:**901-3.

In four patients with acute salpingitis (three of whom also had perihepatitis) the diagnoses were verified by laparoscopy or laparotomy; all four had cultural and serological evidence of current infection with *Chlamydia trachomatis* whereas none had signs of gonococcal infection. Three of the four patients had symptoms in the right upper abdomen, but the liver surface showed signs of perihepatitis in only two. In the fourth patient perihepatitis was diagnosed, although she denied symptoms in the hepatic region. Thus symptoms in the right upper abdomen in a young woman may be an indirect sign of a genital infection.

Authors' summary

Lack of evidence for an association between infection with *Chlamydia trachomatis* and Crohn's disease as indicated by microimmunofluorescence antibody tests

P-A MÅRDH, B URSING, AND E SANDGREN (University of Lund, Sweden). *Acta Pathol Microbiol (B)* 1980; **88:**57-9.

A significant difference has recently been reported in the occurrence of serum antibodies to the lymphogranuloma venereum (LGV) immunotypes of *Chlamydia trachomatis* between patients with Crohn's disease and controls. In the present study, sera from 107 patients with Crohn's disease (33 men and 74 women) were tested for antibodies to *C trachomatis* by an indirect immunofluorescence test using two pools of antigens. Each pool contained antigens to either immunotypes TRIC D-K or LGV 1-3. None of the patients had IgM antibodies to the organism. IgG antibodies occurred significantly more often in female than in male patients. No correlation could be demonstrated between activity and duration of Crohn's disease and the results of the antibody tests.

For comparison, the sera of 50 puerperal women were studied. No difference in the occurrence of IgG antibodies was found between patients and controls when using either of the two pools of antigen, thus indicating that the antibodies detected had probably been induced by TRIC rather than LGV organisms. To conclude, the study did not indicate an association between Crohn's disease and infection with *C trachomatis.*

Authors' summary

A comparison of the sensitivity of immunofluorescence and Giemsa for staining *Chlamydia trachomatis* inclusions in cycloheximide-treated McCoy cells
PE MUNDAY, AP JOHNSON, BJ THOMAS, AND D TAYLOR-ROBINSON (Clinical Research Centre, Harrow, UK). *J Clin Pathol* 1980: **33:** 177-9.

Modulation of *Chlamydia trachomatis* infection by cyclic nucleatides and prostaglandins
ME WARD AND HS SALARI (University of Southampton, UK). *FEMS Microbiol Lett* 1980; **7:** 141-4.

The effect of cortisol treatment on cellular ATP levels and phagocytosis in baby hamster kidney cells infected with *Chlamydia trachomatis*
SI REED AND WD HAHN (Bowling Green State University, Ohio, USA). *FEMS Microbiol Lett* 1980; **7:** 137-40.

Comparison of the proteins and polypeptides of the eight serotypes of *Ureaplasma urealyticum* by isoelectric focussing and sodium dodecyl sulfate polyacrylamide gel electrophoresis
IA SAYED AND GE KENNY (University of Washington, Seattle, USA). *Inter J System Bacteriol* 1980; **30:** 33-41.

Colony morphology, ultrastructure, and morphogenesis in *Mycoplasma hominis, Acholeplasma laidlawii* and *Ureaplasma urealyticum*
GA MELONI, G BERTOLONI, F BUSOLO, AND L CONVENTI (University of Padua, Italy). *J Gen Microbiol* 1980; **116:** 435-44.

Reiter's disease

Reiter's disease after *Salmonella typhimurium* enteritis
JJ WEISS, GR THOMPSON, AND A GOOD (University of Michigan, USA). *J Rheumatol* 1980; **7:** 211-2.

Trichomoniasis

Screening for *Trichomonas vaginalis* infection by use of acridine orange fluorescent microscopy
SS HIPP, MW KIRKWOOD, AND HA GAAFAR (New York State Department of Health, USA). *Sex Transm Dis* 1979; **6:** 235-8.

The acridine orange test for detection of *Trichomonas vaginalis* in smears has been adapted for delayed examination of specimens. Slides sent by post and stained by acridine orange were compared with on-site wet mounts; the acridine orange test detected 96% of all positive results whereas only 76% were detected by wet mounts. In a similar comparison with Papanicolaou smears, the acridine orange test detected 89% compared with 67% detected by Papanicolaou smears.

Authors' summary

Do oral contraceptives inhibit *Trichomonas vaginalis?*
M BRAMLEY AND G KINGHORN (Royal Infirmary, Sheffield, UK). *Sex Transm Dis* 1979; **6:** 261-3.

Sister chromatid exchanges in lymphocytes treated with metronidazole
HC WULF (Finsen Institute, Copenhagen, Denmark). *Dan Med Bull* 1980; **27:** 38-40.

The effect of metronidazole on the number of DNA exchanges between the two chromatids of a chromosome (sister chromatid exchanges, SCE) was studied. SCE evaluation has been reported as a sensitive method of demonstrating the possible mutagenic effects of chemical agents.[1] Leucocytes grown in medium containing metronidazole 30 μg per ml did not show increased SCE over control preparations. However, when the concentration of metronidazole was ≥60 μg per ml, significantly increased SCE were found. This finding is important, as a single 2-g dose of metronidazole produces a plasma concentration of 30-45 μg per ml three hours after ingestion.

A McMillan

Reference

1. Perry P, Evans HJ. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature* 1975; **258:** 121-5.

Candidosis

Phenotypic resistance to miconazole and amphotericin B in *Candida albicans*
EF GALE, AM JOHNSON, D KERRIDGE, AND F WAYMAN (University of Cambridge, UK). *J Gen Microbiol* 1980; **117:** 535-8.

Nutrient-limited yeast growth in *Candida albicans:* effect of yeast-mycelial transition
WM BELL AND WL-J CHAFFIN (Christian University, Texas, USA). *Can J Microbiol* 1980; **26:** 102-6.

Germ-tube induction in *Candida albicans*
MG SHEPHERD, CY YIN, SP RAM, AND PA SULLIVAN (University of Otago, New Zealand). *Can J Microbiol* 1980; **26:** 21-5.

Genital herpes

Indirect immunofluorescence test for detecting type-specific antibodies to herpes simplex virus
T FORSEY AND S DAROUGAR (Institute of Ophthalmology, London, UK). *J Clin Pathol* 1980; **33:** 171-6.

Other sexually transmitted diseases

A comparison of the in-vitro activity of rosamicin, erythromycin, clindamycin, metronidazole, and ornidazole against *Haemophilus ducreyi,* including *β*-lactamase-producing strains
S FELTHAM, AR RONALD, AND WL ALBRITTON (University of Manitoba, Canada). *J Antimicrob Chemother* 1979; **5:** 731-4.

Multicentric, pigmented carcinoma-in-situ in the vulva in association with vulval condylomata acuminata

S LAOHADTANAPHORN, JC HUNTER, AND ID ANSELL (General and City Hospitals, Nottingham, UK). *Aust NZ J Obstet Gynaecol* 1979; **19**:249-52.

A 22-year-old British woman developed pigmented lesions of the vulva three months after having vulval condylomata acuminata. She had had 15 applications of podophyllin and her consort had also had penile warts.

Subsequent multiple biopsies showed the pigmented vulval dystrophic nodules to be carcinoma-in-situ. Hyperplastic epithelium, loss of cellular stratification, and cellular atypia were all present. Melanin pigment was demonstrated in some of the atypical epithelial cells but was found chiefly as melanophages in the underlying upper dermis.

The lesions were all excised and had identical histology to that seen in the initial biopsies. The remaining warts were also removed. However, no viral particles were seen on electron microscopy of the skin. The wound healed without secondary infection and no dyspareunia ensued.

Although no other abnormality was found (including cervical exfoliative cytology) in this rare case, the authors stress the possible association with similar changes in the cervix and vagina. It was essential to maintain prolonged follow up so that changes in all parts of the genital tract could be monitored.

J M Harvey

Pediculosis pubis in a clinic for treatment of sexually transmitted diseases

TA CHAPEL, T KATTA, T KUSZMAR, AND D DEGIUSTI (Wayne State University, Michigan, USA). *Sex Transm Dis* 1979; **6**:257-60.

Serologic markers of hepatitis A and B in acute and chronic liver disease

AJ CZAJA (Mayo Clinic, USA). *Mayo Clin Proc* 1979; **54**:721-32.

Miscellaneous

Pulmonary function in Behcet's syndrome

WV EVANS AND RM JENKINS (Whiston Hospital, Prescot, UK). *Scand J Respir Dis* 1979; **60**:314-6.

The combined use of HLA-B5 and the pathergy test as diagnostic markers of Behcet's disease in Turkey

H YAZICI, Y TUZUN, H PAZARLI, S YURDAKUL, AND A MUFTNOGLU (University of Istanbul, Turkey). *J Rheumatol* 1980; **7**:206-10.

Bacterial colonization of human urethral mucosa. I Scanning electron microscopy

S COLLEEN, H MYHRBERG, AND P-A MÅRDH (University of Lund, Sweden). *Scand J Urol Nephrol* 1980; **14**:9-15.

FORTY-EIGHT HOUR PATIENCE

One tablet nightly for three nights means that your patient's patience only needs to last 48 hours. That's all it takes to clear vaginal candidiasis – twice as conveniently as the old One Two Free.

Canesten®

clotrimazole

New 200mg vaginal tablet now only one a night for three nights

Presentations Canesten is available either as 200 mg vaginal tablets in packs of three with applicator or as 100 mg vaginal tablets in packs of six with applicator. Canesten Duopak contains six 100 mg vaginal tablets with applicator plus 20 g cream containing 1.0% clotrimazole. **Dosage** *Vaginal Tablets 100mg* Two vaginal tablets should be inserted at night for three consecutive days. Alternatively, one vaginal tablet daily for six days. *Vaginal Tablets 200mg* One vaginal tablet should be inserted at night for three consecutive days. *Duopak* Vaginal Tablets 100 mg as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to prevent re-infection. **Side effects** Rarely patients may experience transient local mild burning or irritation. **Basic NHS Costs** 6 x 100 mg vaginal tablets with applicator £2.13; 3 x 200 mg vaginal tablets with applicator £1.99; Duopak £3.47. **Product Licence Numbers** Vaginal tablets 100 mg, PL 0010/0015; vaginal tablets 200 mg, PL 0010/0072; Duopak, PL 0010/0016 & PL 0010/0015. Further information is available from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. ® Registered Trade Mark of Bayer Germany.

Sexually
Transmitted
Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $25.00 outside the United States ($22.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $28.00 in the United States and $30.00 in all other countries.

William O. Harrison, M.D.
American Venereal Disease Association
P.O. Box 22349
San Diego, California 92122 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $25.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases.*

☐ I wish to enter a Non-Member or institutional subscription ($28.00 U.S., all other countries $30.00).
(Mail non-member subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105)

Name ______________________________

____

Address ______________________________

City ____________ State Country ____________ Zip ____________

The combination of
simple language and straightforward
line drawings has made the

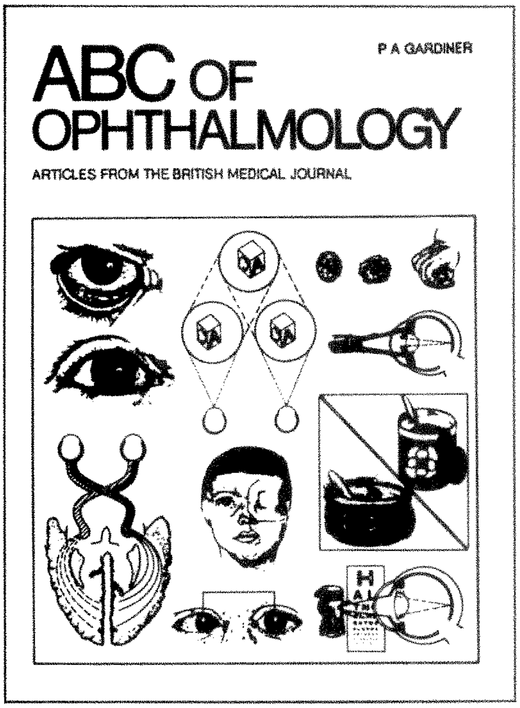
ABC OF
OPHTHALMOLOGY

one of the most popular series
of articles in the Medical Practice section
of the British Medical Journal.

All the common problems are covered - visual defects, squints, glaucoma and cataracts - together with detailed instruction on examination, diagnosis and management.

The articles have now been collected into book form - in exactly the same format as they appeared in the BMJ - price: Inland £2.50; Overseas US$6.25 (Inland £2.00; Overseas US$5.00 for BMA members).

Order your copy now
From: The Publisher,
British Medical Journal,
BMA House,
Tavistock Square,
London WC1H 9JR
or any leading bookseller



P A GARDINER

ABC OF
OPHTHALMOLOGY

ARTICLES FROM THE BRITISH MEDICAL JOURNAL

British Journal of Venereal Diseases August 1980 Vol. 56 No. 4

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Editorial: Gonorrhoea—a continuing problem in the 1980s *page* 193

Sexually transmitted diseases in Nigeria
R D CATTERALL *page* 194

Pleural effusion disease agent as passenger of *Treponema pallidum* suspensions from rabbits
KNUD L FENNESTAD, LIS BRUUN, AND ERIK WEDØ *page* 198

Venereal diseases in the islands of the South Pacific
R R WILLCOX *page* 204

Immunoregulatory properties of serum from patients with different stages of syphilis
J J THOMPSON, R J MANGI, R LEE, AND J M DWYER *page* 210

Immunoglobulin-bearing polymorphonuclear leucocytes in primary syphilis
J D BOS, F HAMERLINCK, AND R H CORMANE *page* 218

Persistent serum immune complexes in syphilis
SIEGFRIED ENGEL AND WOLFGANG DIEZEL *page* 221

Detection of antigonococcal IgA in cervical secretions by indirect immunofluorescence: an evaluation as a diagnostic test
A McMILLAN, G McNEILLAGE, H YOUNG, AND S S R BAIN *page* 223

One-step staining of *Neisseria gonorrhoeae* in urethral discharge by methyl green-pyronin
N F HANNA, D TAYLOR-ROBINSON, G W CSONKA, J R W HARRIS, AND I A AL-SOWAYGH *page* 227

Induction of a cell wall variant of the gonococcus by human amylase
A MELLERSH, S HAFIZ, M G McENTEGART, AND I GEARY *page* 230

Bacterial interference of *Neisseria gonorrhoeae* by α-haemolytic streptococci
M E McBRIDE, W CHRISTOPHER DUNCAN, AND JOHN M KNOX *page* 235

Epidemiology of gonococcal vulvovaginitis among children in the tropics
K O ALAUSA AND A O OSOBA *page* 239

Penicillinase-producing gonococci in the Netherlands
H BIJKERK *page* 243

Gonorrhoea in Rotterdam caused by penicillinase-producing gonococci
K C NAYYAR, R C NOBLE, M F MICHEL, AND E STOLZ *page* 244

Antibiotic sensitivities of gonococci isolated in Rotterdam and results of treatment with cefuroxime
K C NAYYAR, M F MICHEL, AND E STOLZ *page* 249

Sensitivity of *Neisseria gonorrhoeae* to spectinomycin and thiamphenicol
K GRUNDER AND D PETZOLDT *page* 252

HR 756: a new cephalosporin in the treatment of gonorrhoea caused by ordinary and penicillinase-producing strains of *Neisseria gonorrhoeae*
V S RAJAN, E H SNG, R PANG, N J TAN, T THIRUMOORTHY, AND K L YEO *page* 255

Contact tracing in patients with genital chlamydial infection
I THELIN, A-M WENNSTRÖM, AND P-A MÅRDH *page* 259

Confidentiality in the National Health Service and in the service for sexually transmitted diseases
R D CATTERALL *page* 263

Condylomata lata of the palms: an unusual site
M EL-SAAD EL-RIFAIE *page* 267

Correspondence *page* 269

Notices *page* 270

Correction *page* 270

Book reviews *page* 271

Abstracts *page* 272

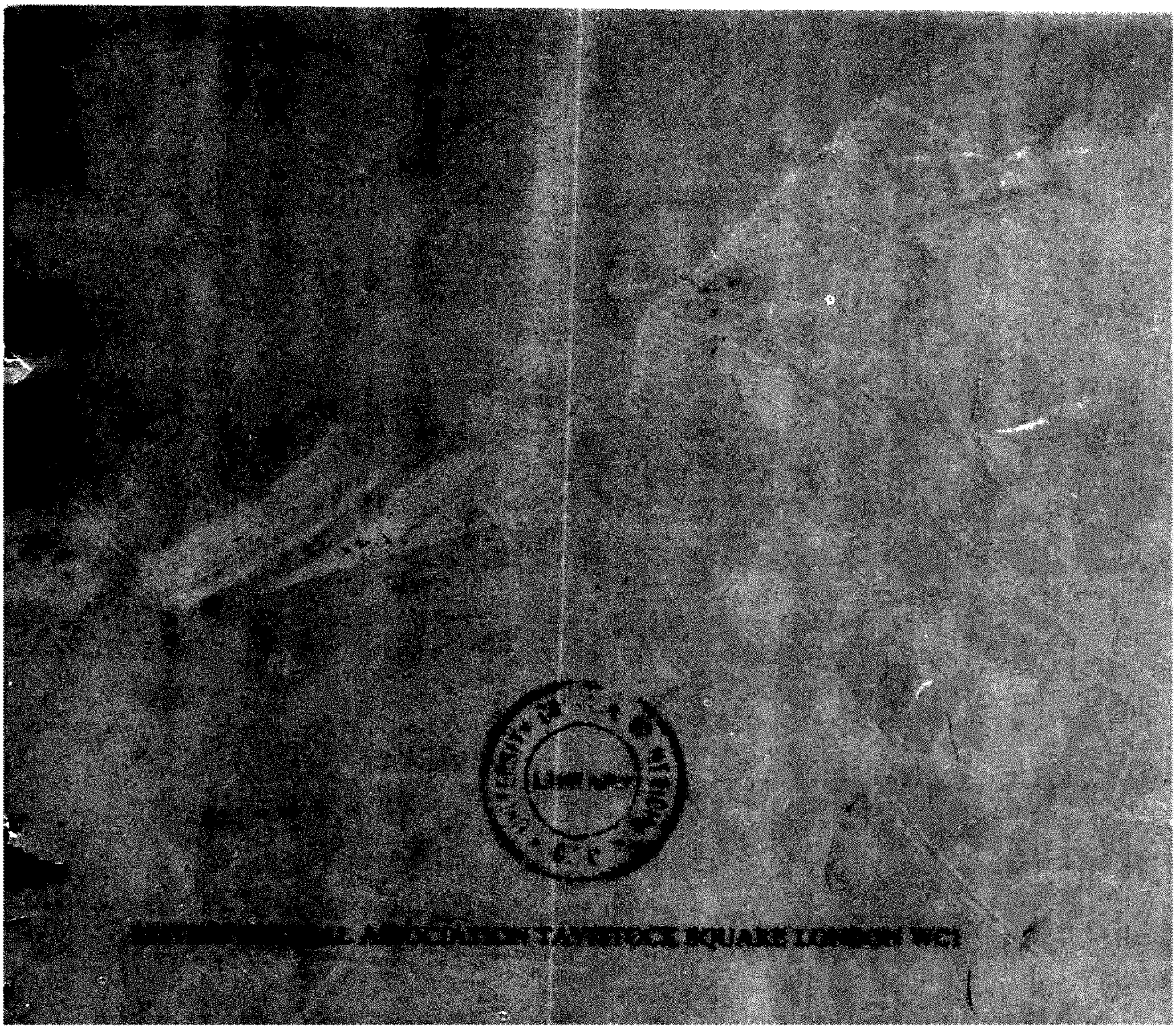
ASTM CODEN: BJVDAK 56 (4)193-276 (1980) ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

Acc.
13.2.81

[illegible]

British Journal of
Venereal Diseases



University [illegible] Library

[illegible] ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

A E WILKINSON
(Editor)

B A EVANS
(Assistant Editor)

A McMILLAN
(Assistant Editor, Abstracts)

C B S SCHOFIELD
(President, MSSVD)

J BARROW
(Hon Sec, MSSVD)

M W ADLER

G CAUSSE

G CSONKA

L M DRUSIN

J M KNOX

G D MORRISON

I PHILLIPS

E STOLZ

R N THIN

J WALLIN

EDITOR,
British Medical Journal

P D SIMMONS
(Indexer)

SHUNA JUDGE
(Technical Editor)

This *Journal,* founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A E Wilkinson, 22 Kingsend, Ruislip, Middlesex HA4 7DA. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals,* available from the Publishing Manager, *British Medical Journal,* BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; **55:** 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS. *Venereal Diseases.* 3rd ed. London: Baillière and Tindall, 1975.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 214-22.

PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £23.00 in the United Kingdom and Republic of Ireland and US\$55.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US\$55.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal,* 1172 Commonwealth Avenue, Boston, Mass. 02134. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases,* BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1980 by the *British Journal of Venereal Diseases.* This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.

For the comprehensive management of non-gonococcal urethritis

Deteclo

Tetracycline hydrochloride, Chlortetracycline hydrochloride, Demeclocyline hydrochloride

for first line therapy

Deteclo will resolve the symptoms of NGU in the majority of cases. One tablet b.d. for 21 days gave 88.6% success in NGU.[1]

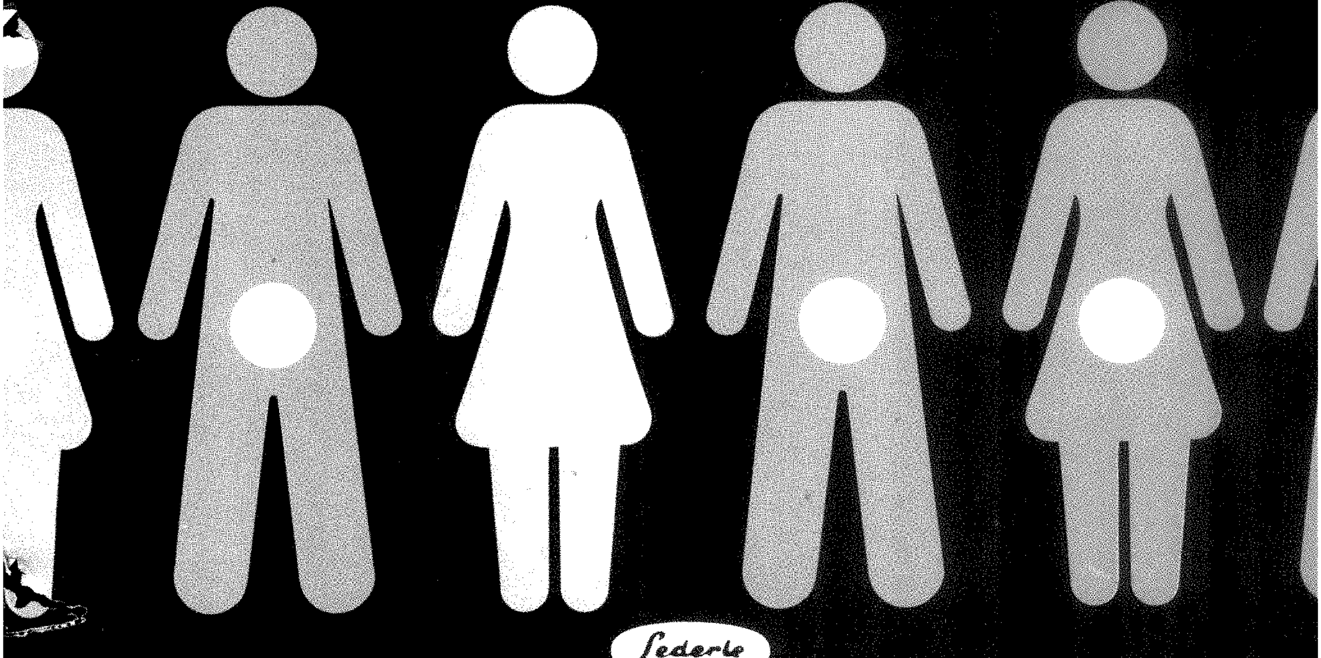
. . . but for the 1 patient in 4 who presents with recurrent NGU, Minocin is the logical choice for rapid resolution of symptoms.

The seven day treatment course, exceptional activity against chlamydia[2] and ureaplasma[3] and the simple b.d. dosage make MINOCIN the treatment of choice in cases of recurrence.

Minocin

Minocycline hydrochloride

for recurrent NGU



Lederle

For Prescribing Information see over page

Deteclo

for first line therapy

Prescribing Information

Uses

Venereal Diseases. Non-Gonococcal Urethritis. Urinary tract infections. Other infections due to DETECLO-sensitive organisms.

Dosage

Adults only.

Non-Gonococcal Urethritis: One tablet twice daily for 10-21 days.

Gonorrhoea: 1200 mg (4 tablets) followed by a similar dose six hours later.

Other Infections: One tablet every 12 hours. This may be increased to three or four tablets daily for short periods in more severe infections.

Contra-indications

Known tetracycline hypersensitivity. Overt renal insufficiency. Systemic lupus erythematosus.

Precautions

Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts. Lower doses are indicated in cases of renal impairment to avoid excessive systemic accumulation, and, if therapy is prolonged, serum level determinations are advisable.

Warnings and side effects

Gastro-intestinal disturbances are the side effects most commonly reported. A yellow to brown discolouration of teeth is a possible sequela of treatment during pregnancy. Photoallergic reactions may occur in hypersensitive persons.

Legal category

POM

Presentation

Each blue 300 mg tablet printed 'Lederle 5422' contains: tetracycline hydrochloride 115.4 mg, chlortetracycline hydrochloride 115.4 mg, demeclocycline hydrochloride 69.2 mg. Supplied in bottles of 100 and 500 tablets at a basic NHS cost of £9.11 per 100 tablets.

(correct at time of going to press)

Product licence number Tablets PL/0095/5070

DETECLO is a Trademark

Minocin

for recurrent NGU

Prescribing Information

Uses

Venereal Diseases. Non-Gonococcal Urethritis. Urinary tract infections. Prostatitis. Salpingitis. Other infections due to MINOCIN-sensitive organisms.

Dosage

Adults only.

Non-Gonococcal Urethritis: One tablet twice daily for a minimum of 7 days.

Gonorrhoea: In adult males, 300 mg as a single dose. Adult females may require more prolonged therapy.

Other Infections: Two tablets initially followed by one tablet twice daily.

Unlike earlier tetracyclines, absorption of MINOCIN is not significantly impaired by food or moderate amounts of milk.

Contra-indications

Known tetracycline hypersensitivity. Systemic lupus erythematosus.

Precautions

Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts.

Warnings and side effects

Gastro-intestinal disturbances are the side effects most commonly reported. Dizziness may occur. A yellow to brown discolouration of teeth is a possible sequela of treatment during pregnancy.

Legal category

POM

Presentation

Orange tablets printed 'Lederle 5334' containing 100 mg of minocycline as the hydrochloride. Supplied in packs of 9 and 45 tablets at a basic NHS cost of £15.78 per 45 tablets.

(correct at time of going to press)

Product licence number Tablets 100mg PL/0095/0006

MINOCIN is a Registered Trademark

Full prescribing information is available on request

Lederle Laboratories
A division of Cyanamid of Great Britain Ltd
Fareham Road, Gosport, Hants PO13 0AS

1. Thambar, I V, *et al*, Brit J Vener Dis 1979; **55** (4): 284–288
2. Bowie, W R, *et al*, J Infect Dis 1978; **138** (5): 655–659
3. Spaepen, M S, *et al*, Antimicrob Ag Chemother 1976; **9** (6): 1012–1018

THEY CALL IT CRABS
You may know it as Phthirus pubis

Whatever you call them, pubic lice are a problem, because any measure you adopt to eradicate them must take care of both adult lice and nits, while being cosmetically acceptable. Quellada Shampoo fills the bill particularly well. Its active constituent, gamma benzene hexachloride, is rapidly lethal to crab lice, while the foaming shampoo base ensures that Quellada penetrates through to the nits. So a single application of Quellada kills lice *and* nits. Quellada Shampoo is easy and pleasant to use, because it doesn't smell or irritate the skin.

Quellada PC Shampoo
gamma benzene hexachloride 1%

kills pubic lice and nits in 4 minutes.

Presentation
Gamma benzene hexachloride 1% in a specially formulated foaming shampoo base.

Uses
Antiparasitic and ovacidal. For use on hairy areas of the body in the treatment of lice infestations, e.g. head lice, body lice, pubic lice.

Dosage and administration
Apply to wet hair, rub vigorously for four minutes, rinse and comb with fine-tooth comb.

Contra-indications, Warnings etc.
Avoid contact with the eyes. Should lather accidentally get into eyes, flush immediately with water. Do not use more than twice in any one infestation nor in any one week. Do not use on broken or infected skin.

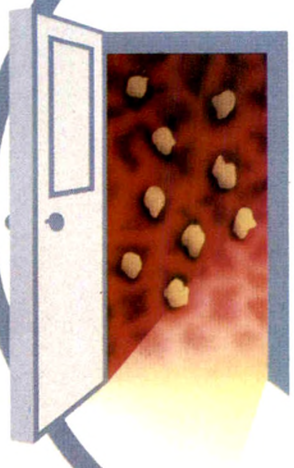
Packaging and price
Bottles of 100 and 500ml. Basic NHS cost 60p for 100ml.

Product Licence No.
0036/5001.

Further information is available on request.

Stafford-Miller Limited,
Professional Relations Division,
Hatfield, Herts. AL10 0NZ.

LIBRARY



In urinary tract infections

New ipral™

trimethoprim 100 mg

...the way to effective treatment of urinary tract infections

Trimethoprim – proved effective as a single entity without the side-effects associated with sulphonamides.

"Trimethoprim alone may well prove as effective as trimethoprim-sulphamethoxazole combinations ..."[1]

96% success in general practice.[2] Half as many side-effects.

Results of 107 Courses of Treatment in 96 Domiciliary Patients

Antibiotic	Overall Success
Ampicillin	89%
Cephalexin	62%
Co-trimoxazole	81%
Trimethoprim	**96%**

ipral simple treatment for acute infections – 2 tablets b.d.

ipral for routine use in chronic U.T.I. – 1 tablet b.d.

In a study on recurrent U.T.I.'s
"... trimethoprim is the most effective of the antibacterial agents examined."[3]

Greater efficacy with fewer side effects.

In the trial in acute U.T.I.'s

"... the total side effects caused by ampicillin, cephalexin and co-trimoxazole were similar and occurred twice as often as when trimethoprim was given alone ..."[2]

Antibiotic	No. of Patients	% Side Effects
Ampicillin	88	22
Cephalexin	84	25
Co-trimoxazole	83	21
Trimethoprim	**84**	**8**
Total	339	

Prescribing information

IPRAL tablets

Each tablet contains trimethoprim B.P. 100 mg.

Indications

Ipral is indicated for the treatment and prophylaxis of acute and chronic urinary tract infections.

Actions

Ipral is active in-vitro against most pathogenic Gram-positive and Gram-negative bacteria. Exceptions include Neisseria species and Pseudomonas aeruginosa.

Dosage and administration

For acute urinary tract infections 2 tablets twice daily is recommended. For long term and prophylactic therapy 1 tablet twice daily is recommended.

Contra-indications

Ipral should not be given to patients with severe renal insufficiency where blood levels cannot be monitored regularly. Ipral should not be used in pregnancy or during the first few weeks of life.

Precautions

Care is necessary in administration to patients with impaired renal function. Special precaution should be exercised in patients with a predisposition to folate deficiency.

Side-effects

Nausea, vomiting and skin rashes may occur but are rare. Ipral may depress haematopoiesis during prolonged therapy. This has proved reversible when treated with calcium folinate.

Treatment of overdosage

Gastric lavage. Calcium folinate may be used to counteract any effect of Ipral on bone-marrow.

Package quantities and price

Packs of 100 tablets £5.30.

Product Licence No. - 0034/0190.

Full prescribing information available from:
Technical Services Department, E. R. Squibb & Sons Ltd.
Regal House, Twickenham, TW1 3QT
Tel: 01-892 0164

5280 Oct 79

Ipral is a Trade Mark of E. R. Squibb & Sons Ltd

...the way to effective treatment of urinary tract infections

- As effective as trimethoprim/sulphamethoxazole combination
- Avoids the side effects of sulphonamides
- At a sensible price

and "Trimethoprim given alone was shown to be much better tolerated."[2]

"Treatment with TMP (trimethoprim) alone is superior to the combined regimen due to a smaller incidence of side-effects; ..." thus "... introduction of TMP alone on the market is recommended."[4]

"Less gastrointestinal side-effects and skin rashes have occurred with trimethoprim ..."[3]

References
1. Lancet 1979, **1**, 493. 2. Br. med. J. 1972, **2**, 673.
3. Ann. clin. Res. 1978, **10**, (suppl. 22). 4. Chemother. 1973, **19**, 314.

New ipral™

trimethoprim 100 mg

more than half the story

SQUIBB™ Full prescribing information available from Technical Services Department, E. R. Squibb & Sons Ltd. Regal House, Twickenham, TW1 3QT. Tel. 01-892 0164

Ecostatin™

econazole nitrate

"Once the infection has been established in the female, it can undoubtedly be transmitted to the male consort...
This may, in turn... serve as a reservoir for the reinfection of his partner after her condition had responded successfully to treatment. The risk of this sexual 'ping-pong' has been recognised..."[1]

the most active fungicide available prevents 'sexual ping-pong'

Just one pessary a night for three nights clears candidal vaginitis. The cream treats the anogenital region, and its simultaneous use by the male partner will prevent re-infection.

- Total pessary disintegration independent of vaginal secretion.
- Ecostatin pessaries melt and disperse within ten minutes extensively coating the vaginal wall.
- Short course of treatment and acceptable presentation leads to improved patient compliance.
- Primary resistance has not been observed.
- Twin pack helps prevent re-infection and can be used to treat the sexual partner.

ECOSTATIN*
Pessaries: Each contain 150 mg econazole nitrate.
Twin Pack: Three pessaries plus 15 g Ecostatin cream. 1% w/w econazole nitrate.

Indications
Pessaries and Twin Pack: Vulvovaginal candidosis.

Actions
Econazole nitrate is a broad-spectrum antifungal agent.

Dosage and Administration
Pessaries and Twin Pack: One pessary to be inserted at bedtime for three consecutive nights. The cream to be applied twice daily to the anogenital area.

Precautions
The pessaries should not be used during the first trimester of pregnancy unless deemed essential for the patient's wellbeing.
Topical preparations should not be used in or near the eyes and used with care in the presence of eczematous dermatitis.

Side-effects
Occasional local irritation. The pessaries may cause transitory discomfort.

Package quantities, prices and Product Licence Numbers
Pessaries: Pack of 3 with applicator – £3.60
PL No. 0085/0013
Twin Pack: 3 pessaries, applicator and 15 g cream – £4.70
—
Cream: 15 g – £1.25; 30 g – £2.50
PL No. 0085/0014

Product Licence Holder
F.A.I.R. Laboratories Ltd.,
179 Heath Road,
Twickenham, TW1 4BJ

(1) Br. J. Sex. Med. 1974, 1, 21.

Further information is available from
FAIR Laboratories Ltd., 179 Heath Road, Twickenham, Middx. TW1 4BJ
**Ecostatin is a registered trademark and FAIR Laboratories is the authorised user.*

FAIR

Br J Vener Dis 1980;**56**:277-81.

Venereal diseases in the Pacific Islands*

Papua New Guinea

R R WILLCOX

From the Department of Venereology, St Mary's Hospital, London

SUMMARY Papua New Guinea, which contains nearly three-quarters of the population of the 20 islands, or island groups, studied by the South Pacific Commission has a commensurate proportion of reported cases of syphilis and gonorrhoea. It is a country with an exceptional interest for the venereologist as it exhibits all the facets of venereal disease problems as experienced the world over. With the opening up of communications syphilis has gained a foothold in what were areas previously endemic with yaws; moreover, some yaws still remains (particularly in the offshore islands)—the two conditions tending to be mutually exclusive. In the area around the capital, Port Moresby, the prevalence of Donovanosis is unparalleled.

Introduction

Venereal diseases in the islands of the Pacific Ocean have already been considered in two previous papers;[1 2] the first related to the islands of the North Pacific and the second to those of the South Pacific. The islands of the latter, of which Papua New Guinea is by far the largest member, provide epidemiological data to the South Pacific Commission.

The territory is separated by the Torres Strait from the tip of Queensland in Australia (figure). Its mountainous hinterland prevents road access from its capital, Port Moresby, in the south-east to the principal towns. A highway has been constructed however across the Highlands from the port of Lae in the east to Mount Hagen and beyond in the west.

Previously the offshore islands of the Bismarck Archipelago and the northern Solomon Isles (Manus, New Britain, New Ireland, Bougainville, and Buka) were included in the territory, but the latter have recently seceded to form the independent republic of North Solomons.

AIR COMMUNICATIONS

From Europe, Papua New Guinea is reached by way of Hong Kong or Manila. Within the country, which has much difficult terrain, air travel is very important and a considerable network has been established; 47 different local centres are served by internal flights of three airlines from Port Moresby, 15 are reached from Lae, and more from other centres, for example, Goroka.

COMPARISON WITH OTHER SOUTH PACIFIC ISLANDS

Dominance in statistical terms

In the epidemiological data compiled by the South Pacific Commission, Papua New Guinea has the dominant position among the 20 island territories. Occupying 85% of the total land mass, it contains nearly three-quarters of the population (table I).[3 4] The number of reported cases of syphilis and gonorrhoea is proportionate to the size of the population, but virtually all cases of yaws now reported in the South Pacific are found in this territory.

TABLE I *Population size and prevalence of gonorrhoea, syphilis, and yaws in Papua New Guinea compared with other South Pacific islands*[3 4]

	Total for South Pacific islands (1978)	*Papua New Guinea*	
		No	*%*
Size (square miles)	204 923	174 880	85·3
Population 1978 (× 1000)	4052·4	2990	73·8
Cases of			
Gonorrhoea	13 657	10 386	76·0
Syphilis	4652	3485	74·9
Yaws	883	880	99·7

---

*Based on a paper presented at a meeting of the Medical Society for the Study of Venereal Diseases, Lübeck, West Germany, May 1979

Address for reprints: Dr R R Willcox, Tideway, Lonsdale Road, London SW13

Received for publication 19 December 1979

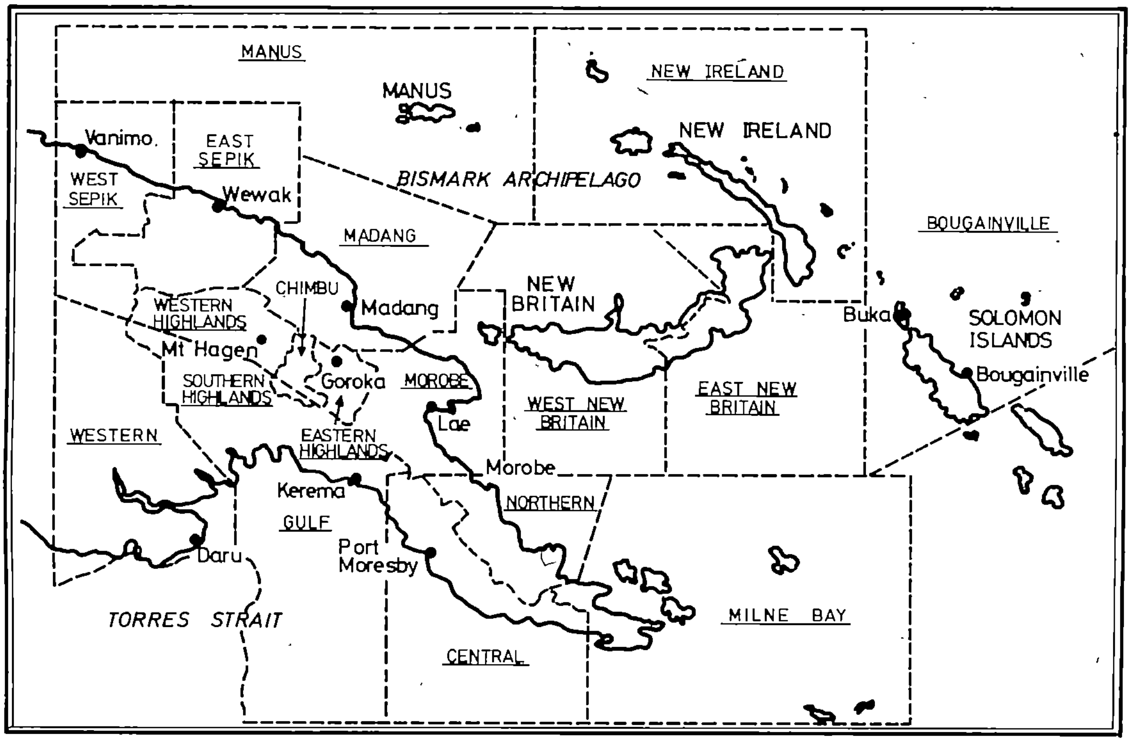


FIGURE *Papua New Guinea showing mainland and island districts*

Population increase
The increasing population of Papua New Guinea and the other islands embraced by the South Pacific Commission is shown in table II. In the five-year period, 1973-78, the estimated population of the whole region increased by 12·8%, but the increase was greater for Papua New Guinea than for the other 19 island groups.[3-6]

GROUPING OF STATISTICS
The statistics of the districts may be conveniently considered in four areas. These comprise (a) the single central district, which includes Port Moresby; (b) the four districts with relatively large populations which straddle the highway through the Highlands (Morobe, Eastern and Western Highlands, and Chimbu); (c) eight other mainly more sparsely populated rural districts on the mainland; and (d) the offshore islands of the Bismarck Archipelago and Northern Solomons. The first two areas have the largest populations and the five island districts the smallest (table III; figure).

Venereal diseases

GONORRHOEA
In the five years, 1973-78, the number of reported cases of gonorrhoea in Papua New Guinea has increased from 202 to 347 per 100 000 whereas that of the other islands (181 per 100 000 in 1978) has remained virtually unchanged (table IV).[4-7]

TABLE II *Population size of Papua New Guinea and other islands of the South Pacific (1973-8)*[3-6]

	Population (× 1000)								
	1973		*1974*		*1977*		*1978*		
	No	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*% Increase (1973-8)*
Papua New Guinea	2620	61·6	2682	61·7	2908	62·3	2990	62·3	14·1
Other islands	1632	38·4	1664	38·3	1763	37·7	1808	37·7	10·8
Total	4252	100·0	4346	100·0	4671	100·0	4798	100·0	12·8

TABLE III *Population of Papua New Guinea according to districts*

Area	No of districts	Population size (× 1000) Estimated (1971)	Per district	% of total
Central district	1	175·5	175·5	7·2
Morobe, Chimbu Eastern Highlands, Western Highlands	4	838·7	209·7	34·4
Other mainland districts*	8	1079	134·9	44·3
Islands†	5	342·3	68·5	14·1
Total	18	2435·5	135·3	100·0

*Northern, Milne Bay, West Sepik, East Sepik, Southern Highlands, Madang, Western, and Gulf
†Manus, East and West New Britain, New Ireland, and Bougainville

In any developing country where the diagnosis may only be clinical, an increase in reported cases does not however necessarily indicate an increase in the true prevalence of the disease but can reflect improved detection and reporting due to better facilities and a greater awareness.

The gonorrhoea figures for Papua New Guinea in 1973 are analysed according to the four areas in table V. By far the greatest number of cases of gonorrhoea (60·1%) was treated in the four districts adjacent to the Highlands highway and only 15·9% in the single Central district, containing the capital. Nevertheless, when expressed as a rate per 100 000 population, the Central district had the highest (462 per 100 000) while the eight mainland districts unconnected with the Highlands highway had the lowest (50 per 100 000). The relatively high rate for the offshore islands was augmented by returns from the port of Rabaul in New Britain and from near and around the large copper mine at Panguna on the island of Bougainville.

TABLE V *Prevalence of gonorrhoea in Papua New Guinea by districts**

Area	No of districts	No of cases	%	Rates per 100 000
Central district	1	811	15·9	462
Morobe, Chimbu, E and W Highlands	4	3069	60·1	366
Other mainland districts	8	535	10·5	50
Islands	5	686	13·4	200
Total	18	5101	100·0	209

*Based on 1973 figures

YAWS

Yaws, which at one time was endemic throughout the islands of the South Pacific, has now been virtually eliminated from all but Papua New Guinea, where—although subjected to mass campaigns in the 1950s—the disease still smoulders on (table VI).

In the area of the South Pacific Commission no less than 1810 (98·6%) of the 1836 cases of yaws reported in the two years, 1977 and 1978, were in Papua New Guinea and only 26 (1·4%) in the other 19 islands (all in the New Hebrides).

Before 1973, fewer cases were returned for Papua New Guinea—that is, 416 in 1972 and 135 in 1971. While such statistics may be related to conscientiousness or enthusiasm in reporting, the recent figures nevertheless suggest a recrudescence from previously low levels. Yaws is still reported in the

TABLE IV *Prevalence of gonorrhoea in Papua New Guinea in relation to other islands of the South Pacific*[4-7]

	1973		1974		1977		1978	
	Cases	Rate*	Cases	Rate*	Cases	Rate*	Cases	Rate*
Papua New Guinea	5156	202	5759	219	8954	308	10 386	347
Other islands	3055	187	2446	147	3308	188	3271	181
Total	8211	116	8205	191	12 262	263	13 657	285

*Per 100 000 population

TABLE VI *Prevalence of yaws in Papua New Guinea in relation to other islands of the South Pacific*[4-7]

	1973		1974		1977		1978	
	Cases	Rate*	Cases	Rate*	Cases	Rate*	Cases	Rate*
Papua New Guinea	954	36	504	19	930	32	880	29
Other islands	16	1	7	0·4	23	1·3	3	0·2
Total	970	23	511	12	953	20	883	18

*Per 100 000 population

rural areas and in the islands of the Bismarck Archipelago, particularly in Bougainville and in New Britain around Rabaul, where more recently small mass campaigns have been undertaken (table VII). The disease is, however, extremely rare in the four Highland districts and nonexistent in the Central district around Port Moresby.

TABLE VII *Prevalence of yaws in Papua New Guinea by districts**

Area	*No of districts*	*No of cases*	*%*	*Rates per 100 000*
Central district	1	0	0	0
Morobe, Chimbu, E and W Highlands	4	4	0·5	0·5
Other mainland districts	8	120	14·1	11
Islands	5	726	85·4	212
Total	18	850	100·0	35

*Based on 1973 figures

While in Bougainville in 1974, from where the greatest number had been reported, I saw some cases of yaws during a tour of some rural health and mission centres. The older nurses expressed no doubts that the disease which they recalled from the 1950s had returned in recent years. It was distinguished from so-called "tropical sores" by its raised white lesions. It is now however a less exuberant condition; whereas formerly it often affected the nose, mouth, buttocks, and soles of the feet, today it usually affects the legs and is often called "dry" yaws. Neither plantar lesions nor bone involvement is now being seen.

It is often stated that syphilis is also becoming a milder disease and doubtless parallel factors exist for both conditions. In the case of yaws, the much expanded schools programme ensures that children with open sores of any kind are more likely to be referred to rural health centres, or to a rural health nurse, much earlier than would previously have been the case.

SYPHILIS

There is no doubt that the incidence of reported syphilis in Papua New Guinea has increased markedly in the five years since 1973 and that this increase has been even more striking in the other 19 islands of the South Pacific Commission (table VIII). Some of this increase however is due to the increasing capability and use of serological screening.

Syphilis has become a problem in the Highlands since a successful yaws campaign between 1957 and 1958. The first effect was noticed in 1970 when 38 cases were reported the previous year.[8] It seemed likely that this rather severe and sudden outbreak reached the Highlands by way of the newly constructed east-west highway, so spreading the disease in a population that had lost its immunity as a result of yaws. As shown by a serological survey of the districts along the Highlands highway in 1970, only four (0·8%) of 506 children under 14 years had reactive results to the *Treponema pallidum* immobilisation test compared with 145 (61·2%) of 237 children the year before on Kar Kar Island, an area with some residual yaws.[9]

Naturally, once interest had been aroused, more and more cases were found. By 1973, nearly 95% of the reported cases of syphilis in the entire country occurred in the four districts adjacent to the Highlands highway (table IX).

TABLE IX *Prevalence of syphilis in Papua New Guinea by districts**

Area	*No of districts*	*No of cases*	*%*	*Rates per 100 000*
Central district	1	37	3·7	21
Morobe, Chimbu, E and W Highlands	4	945	94·7	113
Other mainland districts	8	6	0·6	0·5
Islands	5	10	1·0	3
Total	18	998	100·0	41

*Based on 1973 figures

RELATIONSHIP BETWEEN YAWS AND SYPHILIS

That the environmental and immunological factors which favour the transmission of yaws are not conducive to the spread of syphilis, and vice versa, is well illustrated in Papua New Guinea. In the seven districts in which 98·8% of the syphilis was

TABLE VIII *Prevalence of syphilis in Papua New Guinea in relation to other islands of the South Pacific*[4-7]

	1973		*1974*		*1977*		*1978*	
	Cases	*Rate**	*Cases*	*Rate**	*Cases*	*Rate**	*Cases*	*Rate**
Papua New Guinea	1021	39	1152	43	1937	67	3485	117
Other islands	73	5	327	20	1099	62	1167	65
Total	1094	26	1479	34	3036	65	4652	97

*Per 100 000 population

encountered only four (0·5%) cases of yaws were reported, and in the 10 districts with 99·5% of the cases of yaws only 1·2% of cases of syphilis were reported (table X).

TABLE X *Comparative distribution of reported cases of yaws and syphilis in Papua New Guinea**

District	*No of cases* *Syphilis*	 *Yaws*
Eastern Highlands	277	2
Western Highlands	246	
Chimbu	233	2
Morobe	189	
Central	37	
Northern	3	
Milne Bay	1	
Total (% of national total)	986 (98·8)	4 (0·5)
Bougainville	1	374
East New Britain	2	283
Southern Highlands	2	49
West Sepik		46
West New Britain	1	38
Madang	6	28
Western		12
East Sepik		9
Gulf		4
New Ireland		3
Total (% of national total)	12 (1·2)	846 (99·5)
National total	998	850

*Based on 1972-73 figures

Other sexually transmitted diseases

The other sexually transmitted diseases—for example, nongonococcal urethritis, trichomoniasis, and herpes—are also prevalent but no records of these are available. The most striking feature of these disorders is the very high prevalence of Donovanosis in the Central district, where, in 1973, it was eight times more prevalent than syphilis (a rate of 175 per 100 000, table XI). The reason for the unusually high incidence in this rather localised geographical world area is far from clear but Donovanosis has been prevalent since the first world war, and 25% of 20 000 people in then adjacent West New Guinea were said to be infected in 1925.[10]

TABLE XI *Prevalence of Donovanosis in Papua New Guinea**

Area	*No of districts*	*No of cases*	*%*	*Rates per 100 000*
Central district	1	308	90·3	175
Morobe, Chimbu, E and W Highlands	4	13	3·8	2
Other mainland districts	8	10	2·9	1
Islands	5	10	2·9	3
Total	18	341	100·0	14

*Based on 1973 figures

In this series of three papers I am grateful to the World Health Organisation and the South Pacific Commission for statistical data, to both of these organisations for providing the opportunities to visit the South Pacific area, and especially to the Government of Papua New Guinea for the many kindnesses received during two assignments in that country.

References

1. Willcox RR. Venereal diseases in the islands of the North Pacific. *Br J Vener Dis* 1980;56:173-7.
2. Willcox RR. Venereal diseases in the islands of the South Pacific. *Br J Vener Dis* 1980;56:204-9.
3. South Pacific Commission. *Statistical Bulletin of the South Pacific No 7: Population 1974*. Revised edition, Noumea, New Caledonia: South Pacific Commission, 1977.
4. South Pacific Commission. *Annual Report of the South Pacific Epidemiological and Health Information Service for the Year 1978*. Noumea, New Caledonia: South Pacific Commission, 1979.
5. South Pacific Commission. *Annual Summary of the Infectious Disease Reports of the South Pacific Epidemiological and Health Information Service, 1973*. Noumea, New Caledonia: South Pacific Commission, 1974.
6. South Pacific Commission. *Annual Summary of the Reported Diseases of the South Pacific Epidemiological and Health Information Service, 1974*. Noumea, New Caledonia: South Pacific Commission, 1975.
7. South Pacific Commission. Reported cases of notifiable diseases for the South Pacific Region countries. *Annual Report of the South Pacific Epidemiological and Health Information Service for the Year 1977*. Noumea, New Caledonia: South Pacific Commission, 1978.
8. Rhodes FA, Anderson SEJ. An outbreak of treponematosis in the Eastern Highlands. *Papua New Guinea Med J* 1970;13:49-52.
9. Garner MF, Hornabrook RW. Treponematosis in Papua New Guinea—a review of surveys undertaken between 1964 and 1972. *Papua New Guinea Med J* 1973;16:189-93.
10. Maddocks I. Donovanosis in Papua. *Papua New Guinea Med J* 1967;2:49-54.

Br J Vener Dis 1980;**56**:282-4.

Sexually transmitted diseases in Indonesia

MARWALI HARAHAP

From the Department of Dermatovenereology, University of North Sumatra School of Medicine, Medan, Indonesia

SUMMARY Epidemiological factors and changing ecological conditions have greatly facilitated the spread of sexually transmitted diseases and led to their rising incidence in Indonesia. Gonorrhoea is at present very prevalent, and drug resistance among circulating strains of gonococci is a contributing factor. Despite medical advances in both diagnosis and treatment of sexually transmitted diseases, these are becoming commoner; unlike other communicable diseases they have so far defied efforts to control them.

Introduction

Indonesia is a country of more than 3000 islands with 140 million inhabitants (72 million female and 68 million male). The age distribution indicates a population with a 2·4% growth rate during these last few years. There are 60 million (43%) people between the ages of 15 and 49 years.[1]

The medical needs of the population are cared for by 7000 doctors, 120 of whom are dermatologists and venereologists (a ratio of about one specialist for each specialty to one million inhabitants) (personal communication). Although poverty is still widespread, expanding building programmes and a rising standard of living bear witness to economic advances.

Epidemiology

GONORRHOEA

Sexually transmitted diseases (STDs) in Indonesia are poorly documented, but the figures available suggest an alarmingly high prevalence of gonorrhoea. A considerable proportion of the patients are treated by general practitioners, by private dermatovenereologists, and even by nurses; alternatively, patients simply treat themselves. Almost 80% of the population receive their primary care in peripheral medical units. In most of these units, laboratory facilities are inadequate, so diagnosis has to be made on clinical grounds and its accuracy therefore depends on the ability of the primary physician and medical auxiliaries.

Complications, such as salpingitis, epididymitis, and ophthalmia neonatorum, are also encountered.

SYPHILIS

Syphilis appears to be on the increase in Indonesia but this is less alarming than the increase in gonorrhoea. In 1974, the number of cases of syphilis reported by the provincial health authorities to the Ministry of Health was 483, of which 401 cases were of early, 57 of latent, and 25 of congenital syphilis.[2] In 1977, the number of cases of syphilis reported was 1909.[2]

The true prevalence of syphilis is likely to be much greater, as cases tend to be concealed when they are seen in a wide variety of hospital departments, such as obstetric, paediatric, and dermatological departments, as well as in private practice. One of the causative factors of the increase in syphilis is the loss of protective crossimmunity between *Treponema pallidum* and *Treponema pertenue* infections after the highly effective campaigns to eradicate yaws in the early 1960s.

OTHER STDs

Most of the other sexually transmitted diseases occur throughout Indonesia, but granuloma inguinale and lymphogranuloma venereum are rarely seen. The former was endemic before the second world war in the southern part of Irian Jaya. Since the war it has appeared in Java but is now rare. Lymphogranuloma venereum is also uncommon.

Non-specific urethritis is not yet widely recognised because of the preoccupation with gonorrhoea and poor diagnostic facilities.

Address for reprints: Dr M Harahap, Department of Dermatovenereology, School of Medicine, University of North Sumatra, Medan, Sumatra, Indonesia

Received for publication 13 August 1979

Factors underlying rising incidence

ECONOMIC AND CULTURAL

Contact between previously isolated rural societies and the outside world is increasing as a result of economic development, and STDs are being introduced in its wake.

The most important factors of all however have been increasing urbanisation and industrialisation. Young adults of both sexes are migrating to the towns in great numbers in search of work and a more exciting life. As a result transition from a traditional society to urban life, family disruption, housing problems, and loneliness all contribute to altered codes of behaviour and to promiscuity. The changing demographic picture is a result of the high birth rate, despite the intensified family planning programme, and an increase in the most sexually active and susceptible section of the population. The discarding of traditional values, the disappearance of previous restraints—such as religion and family and public opinion—have all been conducive to permissiveness. A substantial rise in tourism and occupational travel has contributed most to the increasing number of cases.

PROSTITUTION

Prostitution still plays an important part in the spread of STDs in Indonesia. Prostitutes account for 80-90% of the sources of infection of male patients treated for STDs. The prostitutes are examined every two weeks, when cervical and rectal swabs are cultured. Serological tests for syphilis are carried out every three months, and appropriate treatment is given when the results are positive. Between 10 and 60% of prostitutes have gonorrhoea at initial culture. Circulating antitreponemal antibodies are rare among these prostitutes (Harahap *et al,* paper read at 14th Congress of Medicine, Singapore, 1979). The prevalence of oropharyngeal gonorrhoea among prostitutes is between 5 and 18·2%.[3]

Mobility among prostitutes is common; they move freely from one brothel to another or between the brothel and the non-registered sectors. Moreover, these prostitutes may move to neighbouring countries, such as Singapore and Malaysia, and back to Indonesia. Periodically, prostitutes are arrested by the police so that they can be examined and treated.

Homosexuality does not play an important role in the transmission of disease in Indonesia.

EDUCATION

Lack of understanding of the nature of STDs may encourage its spread. However, knowledge alone is not enough since high STD rates have been reported among university students who are well informed about STDs. Most students obtain their information not from instruction but from their own studies of books and from radio, television, and the press.

OCCUPATION

STDs are an occupational hazard among certain occupational groups—such as taxi and truck drivers, salesmen, soldiers, and sailors—as well as among bar girls and prostitutes.

ASYMPTOMATIC CARRIERS

Asymptomatic carriers are an important source of infection. Over half the women infected with gonorrhoea have no symptoms unless complications ensue; hence without treatment they constitute a reservoir of infection. A proportion of the men may also be asymptomatic carriers, particularly in high-risk groups (Harahap *et al,* paper read at 14th Congress of Medicine, Singapore, 1979).

PENICILLINASE-PRODUCING GONOCOCCI

Penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strains have been reported from the Philippines, Malaysia, Singapore, and Thailand.[4] Although individuals and groups of people are increasingly mobile within and between countries for cultural, commercial, leisure, and occupational reasons, so far PPNG strains have not been observed in Indonesia. Tests have been performed by some laboratories.

PENICILLIN RESISTANCE

Partial resistance to penicillin has developed as a result of inadequate treatment regimens. Some failures are attributable to the administration of penicillin preparations with delayed absorption and prolonged low serum concentrations.

With the plate dilution technique, minimum inhibitory concentrations (MICs) for gonococci isolated during trials and in treatment failures have shown that the organism has become increasingly resistant to penicillin and the tetracyclines (Soendjojo *et al,* paper read at 3rd Conference of Dermatology, Bali, Indonesia, 1978).[5] Gonococcal strains which have a MIC of penicillin greater than 0·075 μg/ml are considered to be less sensitive strains.

In a study conducted in Medan in 1977, 29 gonococcal isolates were tested for antibiotic sensitivity to penicillin; of these, 79% were less sensitive.[5] In Surabaya, in 1978, 83% of 42 strains were found to be less sensitive (Soendjojo *et al,* paper read at 3rd Conference of Dermatology, Bali, Indonesia, 1978).

Chemotherapy

Penicillin was first introduced for the treatment of syphilis and gonorrhoea in Indonesia in 1946, just after the second world war. An intramuscular injection of procaine penicillin 4·8 megaunits with probenecid 1 g by mouth or ampicillin 3·5 g with probenecid 1 g by mouth are at present given routinely for the treatment of uncomplicated gonorrhoea. These dosages will have to be increased in the near future.

In Indonesia, the high percentage of strains which are less sensitive to tetracycline have led to a reluctance to use it in the treatment of gonorrhoea.

Single intramuscular doses of spectinomycin 2 g or kanamycin 2 g are still the drugs of choice for patients infected with less sensitive strains or with penicillinase-producing gonococci. However, the widespread use of spectinomycin and kanamycin may produce resistance among strains of *N gonorrhoeae.* Spectinomycin also has the disadvantage of being relatively expensive. The general replacement of penicillin by spectinomycin would be very costly. One of the possible consequences of the wide use of spectinomycin is an increase in the prevalence of infectious syphilis.[4]

The recommended treatment for early infectious syphilis is benzathine penicillin 2·4 megaunits given as a single dose. For patients who are sensitive to penicillin, tetracycline or erythomycin 2 g daily for 15 days is given.

Conclusions

We need to recognise the limitations of the present measures used to control STDs and to accept that, if we want control to the point of eradication, the only hope lies in immunisation.

Addendum

In June 1980 Widjaja *et al* (paper read at the Fourth Regional Conference of Dermatology, Penang, June 1980) reported four cases of gonorrhoea due to penicillinase-producing strains of *N gonorrhoeae* in Jakarta. Two methods were used to test for penicillinase production—namely, the iodometric and the acidometric tests. All isolates had MICs of $\geqslant 16$ μg/ml for penicillin G, of 2-4 μg/ml for tetracycline, and of 10-24 μg/ml for spectinomycin.

References

1. Population Reference Bureau. *Data on World Population* Gajah Mada: Institute of Population, University of Gajah Mada, 1977.
2. Ministry of Health Subdirectorate for Diseases Control: Framboesia and Venereal Disease. *Reported Number of Syphilis and Gonorrhoea Cases,* 1978. Republic of Indonesia· Ministry of Health, 1978.
3. Sastrowidjojo H, Idajadi NA, Harijanto NSR, Dan Mochny IS. Pharyngitis gonorrhoeica. In: *Proceedings of the Second National Congress of Dermatovenereology.* Surabaya: Airlangga University Press, 1978.
4. Perine PL, Morton RS, Piot P, Siegel MS, Antal GM. Epidemiology and treatment of penicillinase-producing *Neisseria gonorrhoeae. WHO/VDT/79.429. WHO/VDT/RES/GON/79.122,* 1979.
5. Hutapea N. Sensitivitas strain gonococci yang diperoleh dari para WTS dari lokalisasi T3 Belawan, Medan. In: *Proceedings of the Second National Congress of Dermatovenereology* Surabaya: Airlangga University Press, 1978.

Br J Vener Dis 1980;**56**:285-90.

Mechanisms of selective toxicity of metronidazole and other nitroimidazole drugs

DAVID I EDWARDS

From the Chemotherapy Research Unit, Department of Paramedical Sciences, North-east London Polytechnic, London

SUMMARY The selectively toxic effect of nitroimidazole drugs towards anaerobic bacteria and protozoa depends on a number of factors. The killing action of such drugs as metronidazole requires the reduction of the nitro group, a process which influences the rate of entry of the drug into the susceptible cell and which is determined by mechanisms involving ferredoxin-linked (or the equivalent) reactions in the cell. The reduced agent subsequently causes strand breakage of DNA, the extent of which depends on the A + T content of the DNA. Other effects of such drugs may include the possible inhibition of DNA repair mechanisms which exacerbate DNA damage. Inhibition of activity of nitroimidazoles may be caused by aminothiol radical scavengers and radioprotectors normally present in the cell or by the presence of other organisms in the environment (that is, the vagina) capable of inactivating the drugs.

Introduction

In 1978, an editorial in this journal[1] gave a short review of the types, availability, and relative activity of nitroimidazoles used primarily against *Trichomonas vaginalis* infections. It is now timely to review the mechanism of action of such drugs in an attempt to explain their selective action and their unique contribution to the chemotherapy of anaerobic organisms since their introduction in 1959.

The first of these drugs, metronidazole, has been studied more widely than any other and consequently will be used as the type example. Metronidazole (1-β-hydroxyethyl-2-methyl-5-nitroimidazole) is effective against a variety of protozoal diseases including trichomoniasis,[2] giardiasis,[3] amoebiasis,[4] and balantidiasis,[5] as well as being a valuable agent for the treatment of bacterial disease caused by anaerobes—for example, Vincent's disease[6] and post-operative infections.[7] [8] More recently, much interest has been shown in its possible application as a specific radiosensitiser of hypoxic tumours together with the 2-nitroimidazole, misonidazole.[9-13]

Other nitroimidazole drugs, such as tinidazole, sulnidazole, secnidazole, and ornidazole, have broadly similar spectra of activity but none has sufficient advantages to supplant metronidazole from its role as the drug of choice for many bacterial and protozoal diseases. The structure of the drugs is shown in the figure. The spectrum of activity of nitroimidazoles is unique in that unlike any other antimicrobial drug they encompass Gram-positive and Gram-negative bacteria, protozoa, and even a few nematode worms, all of which are either anaerobic or micro-aerophilic. Thus the selective toxicity of these drugs rests on their ability to exert a cytotoxic effect specific to anaerobes.

Mode of action

The first studies on the mode of action of metronidazole indicated that it inhibited the output of H_2 gas from *T vaginalis* before that of CO_2, which parallels cell death, indicating that H_2 inhibition is a primary effect.[14] Further studies showed that H_2 output was by a phosphoroclastic mechanism of the clostridial type, in which pyruvate and phosphate are converted to acetylphosphate, CO_2, and H_2, and that nitroimidazoles act by accepting electrons from an electron transport protein which is normally involved in H_2 gas evolution.[14] [15] Since other drugs, for example, dimetridazole and tinidazole, have the same effect this inhibition appears to be a general property of 5-nitroimidazoles. However, the impaired function of the phosphoroclastic mechanism is not

Address for reprints: Dr D I Edwards, Chemotherapy Research Unit, Department of Paramedical Sciences, North-east London Polytechnic, Romford Road, London E15 4LZ

Received for publication 7 December 1979

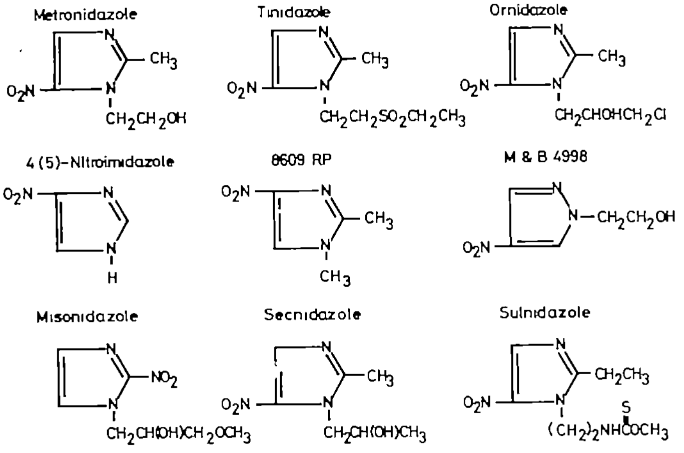


FIGURE *Structures of nitroimidazole drugs. Metronidazole, tinidazole, ornidazole, secnidazole, and sulnidazole are 5-nitroimidazoles, and carnidazole is the 2-methyl congener of sulnidazole. Misonidazole is a 2-nitroimidazole, 8609 RP, a 4-nitroimidazole, and M & B 4998, a 4-nitropyrazole.*

responsible for death since the cell recovers once all the drug is reduced.[16]

Examination of the reduction process showed that the key step lies in the potential of the redox couple between the drug and the electron transfer protein, which in *Clostridia* is ferredoxin. The redox potential of such a reaction is about −450 mV. These low potentials are not found in aerobes and consequently are one factor for the selective toxicity of nitroimidazoles towards anaerobes alone. It follows that any ferredoxin-linked system of the correct potential should be capable of reducing nitroimidazoles and causing cell death. This certainly occurs even with photosynthetic systems where it has been shown that metronidazole inhibits sugar synthesis in sugar cane leaves as a consequence of inhibiting photosystem 1 (where ferredoxin is involved) but has no effect on photosystem 2, the Hill reaction.[17] Further studies in spinach chloroplasts showed a direct inhibition of ferredoxin-linked nicotinamide adenine dinucleotide phosphate (NADP) reduction and corroborative studies have shown that although metronidazole has no effect on the chemo-organotrophic growth of *Rhodopseudomonas acidophila* in the dark, it kills it when grown photo-organotrophically in the light—that is, when ferredoxin-linked systems become operative. Only in ferredoxin-operative systems, or their equivalent, are nitroimidazoles reduced.[15 18]

These events in all susceptible cells have two important consequences. The first is that reduction of nitroimidazoles creates a favourable concentration gradient in which unreduced drug enters the cell relatively rapidly as the internal concentration is decreased due to reduction. This observation, first suggested by Ings *et al*,[19] implies that entry into aerobic cells is more difficult than into anaerobes and is consequently a further factor in explaining the selective toxicity of nitroimidazoles. The second is that reduction of the nitro group is a necessary function of the drugs' cytotoxic action.

The question remains however: if reduction of the drugs via the phosphoroclastic system is not the lethal event in the cell, what is? It becomes a logical hypothesis to propose that the biochemical lesion is caused not by the original drug molecule but by a reduction product or products which act at another site. The reduction of nitroimidazoles is merely an activation mechanism therefore which only anaerobes can carry out.

Site of action

Although protein has been suggested as a possible site of action of these drugs on the basis that about 30% of labelled metronidazole binds to intracellular components, the distribution of which parallels that of protein,[20] no evidence has been published to suggest that any important protein system is inhibited. Instead, evidence has accumulated over the last few years since the suggestion of Ings *et al*[19] that DNA is the major site of action of nitroimidazoles.

Ings' initial observations showed that DNA synthesis in *T vaginalis* was inhibited as measured by incorporation of ^{14}C-labelled thymidine into perchlorate-precipitable material in both the protozoan and in *Clostridia*. This work was subsequently corroborated and extended in our laboratory and showed that DNA synthesis was not only inhibited but existing DNA was broken down in *Clostridia*, and DNA could not be extracted from drug-treated *Trichomonas* or *Clostridia*, indicating that nitroimidazoles cause degradation of DNA.[21] In this study it was apparent that metronidazole was a more potent degrader of DNA than tinidazole even though the latter acted sooner as a consequence of its ability to penetrate cells faster than metronidazole. Significant progress in the study of nitroimidazoles came in 1977, when it was found that chemically reduced metronidazole caused destabilisation of the DNA helix and strand breakage in vitro thus enabling model systems to be used.[22] This latter work however could be criticised on the grounds that the reducing agent itself (dithionite) could cause DNA strand breakage thus making specific measurement of the drug's action difficult.[23]

To overcome the problems of chemical reduction of nitroimidazoles we have developed an electrolytic reduction technique which enables the effect of reduced nitroimidazole on DNA to be measured directly.

Mechanism of action

Polarography of nitroimidazoles enables an assessment of the redox potentials of the drugs to be measured. These potentials can then be used to select the voltage for drug reduction ensuring that it is relevant to those potentials found in anaerobic organisms. Previous studies have already shown that if DNA is added to previously reduced drug there is no effect, indicating that the reduced intermediate responsible for DNA damage is short-lived with a half-life of less than 1 second. If electrolytic reduction is carried out in the presence of DNA at voltages not exceeding minus 1·1-1·2V, which causes DNA denaturation,[24 25] a number of techniques can be used to assess DNA damage. Generally, damage only occurs under anaerobic conditions—namely, an atmosphere of N_2—and as a consequence of the reduction of the nitro group of the drug, which can be monitored by measuring the decrease in the absorption maximum of each drug due to the nitro group.[26]

Recently, studies have been carried out on a variety of nitroimidazoles and nitropyrazoles and the damage to DNA characterised. The major techniques used in this type of study are viscometry of DNA solutions; measurements of the melting and renaturation profiles and its Tm value* using the Rowley equation,[27] measurement of the single-strand content of DNA using hydroxyapatite chromatography, measurement of the decrease in molecular weight (and thus length) by acridine-impregnated agarose gel electrophoresis, and finally the assessment of single-and double-strand breaks in DNA by sucrose gradient sedimentation ultracentrifugation.

Such techniques have shown that reduced drugs decrease the viscosity of DNA (which is a general index of damage), decrease the Tm, and increase the melting range (indicating a decrease in the stability of the molecule and an increase in its polydispersity) and inhibit renaturation (indicating loss of helix integrity). This loss of structural integrity of DNA may be attributed to a number of possible events, but the most obvious is that of scission of the phosphodiester backbone—that is, strand breakage. Confirmatory evidence for strand breakage comes from studies which show a drug-induced increased single-strand content of DNA, its decreased molecular length and weight, and direct measurement of single- and double-strand breaks by sucrose gradient sedimentation.[27-30] These effects have been observed with metronidazole, misonidazole, 4-nitroimidazole, M & B 4998 and 8609 RP (for structures see figure). The overall mechanism of action of all these drugs therefore is to decrease the stability and integrity of DNA by strand breakage.

Metronidazole and misonidazole are presently being evaluated as radio-sensitisers of hypoxic tumours,[31] and the process of radiosensitisation has been extensively studied. This process has many features in common with the cytotoxic properties of nitroimidazoles, including the parameters of nitro-group reduction and DNA strand breakage. Where the processes appear to differ is in the speed and temperature dependence. Thus radiosensitisation is a fast process (about 10-30 milliseconds) as is radiation damage (10 milliseconds) compared with radioprotection mechanisms (850 milliseconds) and cytotoxic effects (about 1 second) and is temperature-independent, whereas cytotoxicity is relatively slow and temperature-dependent. It is now well established that the damaging agent of nitroimidazoles as radiosensitisers is the radical anion ($-NO_2^{\cdot}$) since the efficiency of radiosensitisation of nitroimidazoles can be correlated with their one-electron redox potentials.[32 33]

However, if the efficiency of strand-breakage of DNA in an electrolytic reduction in-vitro system is compared with the one-electron redox potential or the polarographic half-wave potential no obvious correlation exists[27] indicating that the cytotoxic effect towards anaerobes is not due to the nitro radical anion. Recent observations in our laboratory[30] show that reduced nitroimidazole-induced damage of DNA can be prevented by certain aminothiols, notably cysteamine and cystamine, and that cysteamine acts as a radical scavenger whereas cystamine does not but behaves as a radioprotector.

---

*When DNA is heated it is converted from its normal double-stranded form (helix) to single strands (random coil) and this process is accompanied by an increase in absorbance. The midpoint of this helix-coil transition is known as the "melting" or Tm value and is expressed in degrees celsius.

This leads to the conclusion that the reduced agent responsible for DNA damage is at least further reduced beyond the radical anion stage. Precisely what this agent is has not been established yet because of its (their) inherent instability and short half-life. However, the implications of this cytotoxic agent are important for the anticancer action of the drugs since it has been established that the killing effect of the drugs in conjunction with radiation is greater than can be accounted for by radiosensitisation alone. The evidence for this comes from studies in which both normal (oxic) and hypoxic tumour cells are killed.[34] Since the radiosensitisation effect occurs only in hypoxic cells the killing of oxic cells can only be accounted for by a cytotoxic mechanism which does not involve free radicals.

The model electrolytic reduction system is applicable therefore for the study of both the mechanisms of cytotoxicity in anaerobes and in hypoxic cells.

Molecular basis of selective toxicity

Studies of the relative damage done by a number of nitroimidazoles results in two important characteristics of drug action. The first is that the amount of damage varies with the base composition of DNA and the second is that the amount of protection afforded by cysteamine depends on the redox potential of the drug. This latter point is important since the efficiency of action on DNA depends on the degree of strand breakage and aminothiol protection within the cell.

As regards the base composition of DNAs, it can be shown that damage is directly proportional to the A + T content irrespective of what technique is used to measure DNA damage (Edwards *et al*, unpublished observation) and that, using a variety of chromatographic techniques, it appears that thymidine phosphates are specifically released from DNA (Knox *et al*, unpublished observations). These results also argue against a free radical being responsible for such selective DNA damage. Precisely how specific thymidine phosphate release is achieved is not known and will require the elucidation of the active intermediate before this process is understood, but it does enable a molecular basis of selective toxicity to be formulated. Thus, one would expect nitroimidazoles to be particularly selectively toxic to anaerobic organisms which have A + T-rich DNAs and somewhat less so in G + C-rich organisms. The protozoans, *T vaginalis* and *Entamoeba histolytica*, have A + T contents of 71% and 62-78% respectively[35-38] and *Bacteroides* have A + T contents of about 59-61%. These organisms are the main causes of disease for which nitroimidazole drugs have major clinical application. In contrast, ***Rhodospirillum*** and ***R acidophila*** have relatively low A + T contents (38% and 33% respectively) and these organisms are about 25 times less susceptible to nitroimidazoles than the protozoa or bacteria based on relative MICs. The implications of this will be discussed in detail elsewhere, but it is obvious that the particular effectiveness of such A + T-specific drugs against A + T-rich DNA is highly germane to their spectrum of activity or taxonomic specificity.[15]

Resistance and drug inactivation

There have been many reports of trichomonads, including *T vaginalis*, which are resistant to metronidazole or other nitroimidazoles or both.[39-41] Most of these reports are either of no clinical significance, because resistance levels are well below therapeutically obtainable concentrations, or the result of non-anaerobic conditions.[40 41] Because the presence of O_2 in cultures raises the redox potential to positive values drug reduction does not occur and MIC values will increase, erroneously indicating the presence of resistant organisms. The underlying biochemical mechanisms for this may involve carboxylation reactions in *T vaginalis*.[42] However, truly resistant organisms have been developed in the laboratory and these are cross-resistant to a wide variety of nitroimidazoles.[39]

It is unusual that no resistant organisms of clinical significance have arisen during 20 years' use with either metronidazole or any other nitroimidazoles. This would suggest that a single-gene change conferring resistance is itself lethal and indicative of a mechanism and site of action where gene mutation is not compatible with cell survival. However this does not necessarily mean that resistance to nitroimidazoles is impossible; indeed several reports indicate that resistance to nitroimidazoles is possible but not to an extent that would have any clinical significance. The major, albeit rare, occurrence is that of drug inactivation which manifests itself during treatment. In the case of *T vaginalis*, the organism from refractory patients is invariably found to be sensitive in vitro. The reason for this is the presence of other organisms in the vagina, notably *Streptococcus faecalis*, *Escherichia coli*, *Klebsiella*, and *Mimae*, some of which can alone, or in combination, absorb sufficient amounts of drug to lower its effective concentration in the environment below that required for therapeutic purposes. Recent studies have shown that normally aerobic organisms are capable of absorbing amounts of metronidazole without their viability being significantly affected both in aerobic and anaerobic environments.[43,44] This inactivation is a feature both of 5- and 2-nitroimidazoles and may well be relevant to inactivation in other clinically relevant situations, for example, anaerobes in pus.

Clearly, because of the molecular basis of action of nitroimidazoles, resistance could not occur by way of an altered DNA or enzymes involved in DNA biosynthesis. Theoretically however there are mechanisms by which resistance to nitroimidazoles could be accomplished. Since entry into the cell is a prerequisite for drug activation (by reduction) an alteration in membrane permeability to exclude nitroimidazoles could lead to resistance. Attractive though this mechanism may be it must be remembered that nitroimidazoles are small molecules which enter the cell passively and there is no competition for uptake by other imidazoles, for example, histidine, histamine (Edwards, unpublished observations). Any alteration in membrane permeability to exclude nitroimidazoles could well also prevent uptake of histamine or histidine and other small molecules and be lethal to the cell.

A more logical mechanism of resistance would be based not on entry to the susceptible cell but on non-activation of the drug, that is, no reduction of the nitro group. Since the mechanism for reduction lies in the pyruvate phosphoroclastic reaction one would expect a mutation in one of the enzymes of this complex such that the nitroimidazole is not accepted as a substrate. It must be pointed out that resistance to nitrofurans occurs by way of an altered nitroreductase enzyme and that these drugs are not reduced via a ferredoxin-linked mechanism because their redox potential is too positive.[45,46]

It is of great interest that Britz and Wilkinson[47] have recently produced metronidazole resistance in *Bacteroides* using powerful mutagens, but the treated organisms have very much reduced levels of pyruvate dehydrogenase, a key enzyme involved in the phosphoroclastic reaction. Although no resistance mechanisms of this type in protozoa have been reported there have been reports of metronidazole-resistant *Bacteroides* resulting from long-term therapy,[48] and it would be interesting to discover the precise mechanism of resistance of those organisms which are resistant to clinically significant concentrations of drug.

My thanks are due to the Medical Research Council, the Science Research Council, and the Cancer Research Campaign for financial support.

References

1. Anonymous. The nitroimidazole family of drugs. *Br J Vener Dis* 1978;**54**:69-71.
2. Cosar C, Julon L. Activité de l'(hydroxy-2-ethyl)-l-methyl-2-nitro-5-imidazole (8823 RP) vis-à-vis des infections experimentales à *Trichomonas vaginalis*. *Ann Inst Pasteur* 1959;**96**:238-41.
3. Powell SJ, McLeod I, Wilmott AJ, Elsdon-Drew R. Metronidazole in amoebic dysentery and hepatic liver abscess. *Lancet* 1966;**ii**:1329-31.
4. Khambatta RB. Metronidazole in giardiasis. *Ann Trop Med Parasitol* 1971;**65**:487-9.
5. Garcia-Leverde A, de Bonilla L. Clinical trials with metronidazole in human balantidiasis. *Am J Trop Med Hyg* 1975;**24**:781-3.
6. Davies AH, McFadzean JA, Squires S. Treatment of Vincent's stomatitis with metronidazole. *Br Med J* 1964;**i**:1149-50.
7. Willis AT, Ferguson IR, Jones PH, *et al.* Metronidazole in the prevention and treatment of *Bacteroides* infections in gynaecological patients. *Lancet* 1974;**i**:1540.
8. Willis AT, Ferguson IR, Jones PH, *et al.* An evaluation of metronidazole in the prophylaxis and treatment of anaerobic infections in surgical patients. *J Antimicrob Chemother* 1975;**1**:393-401.
9. Foster JL, Willson RL. Radiosensitization of anoxic cells by metronidazole. *Br J Radiol* 1973;**46**:234-5.
10. Deutsch G, Foster JL, McFadzean JA, Parnell M. Human studies with "high dose" metronidazole, a non-toxic radiosensitizer of hypoxic cells. *Br J Cancer* 1975;**31**:75-80.
11. Urtasun RC, Chapman JD, Band P, Rabin H, Fryer C, Sturmwind J. Phase I study of high dose metronidazole. An in-vivo and in-vitro specific radiosensitizer in hypoxic cells. *Radiology* 1975;**117**:129-33.
12. Urtasun RC, Band P, Chapman JD, Feldstein ML, Mielke B, Fryer C. Radiation and high dose metronidazole in supratentorial glioblastomas. *N Engl J Med* 1976;**284**:1357-64.
13. Dische S, Adams GE. Use of a hypoxic cell sensitizer misonidazole to improve radiotherapy. In: Siegenthaler W, Luthy R, eds. *Current Chemotherapy*. Washington: American Society for Microbiology, 1978: 1191-2.
14. Edwards DI, Mathison GE. The mode of action of metronidazole against *Trichomonas vaginalis*. *J Gen Microbiol* 1970;**63**:297-302.
15. Edwards DI, Dye M, Carne H. The selective toxicity of antimicrobial nitroheterocyclic drugs. *J Gen Microbiol* 1973;**76**:135-45.
16. O'Brien RW, Morris JG. Effect of metronidazole on hydrogen production in *Clostridium acetobutylicum*. *Arch Microbiol* 1974;**84**:225-33.
17. Edwards DI, Schoolar AI. Inhibition of sugar synthesis and potentiation of chlorophyll degradation in sugar cane leaf tissue by metronidazole. *Z Pflanzenphysiol* 1971;**64**:73-4.
18. Edwards DI, Mathison GE, Platt DJ. Metronidazole—an antimicrobial drug which inhibits photosynthesis. *Z Pflanzenphysiol* 1974;**71**:424-7.
19. Ings RMJ, McFadzean JA, Ormerod WE. The mode of action of metronidazole in *Trichomonas vaginalis* and other organisms. *Biochem Pharmacol* 1974;**15**:1421-9.
20. Muller M, Lindmark DG, McLaughlin J. Mode of action of metronidazole on anaerobic micro-organisms. In: Finegold SM, ed. *Metronidazole*. Amsterdam: Excerpta Medica 1977:12-9.
21. Plant CW, Edwards DI. The effect of tinidazole, metronidazole and nitrofurazone on nucleic acid synthesis in *Clostridium bifermentans*. *J Antimicrob Chemother* 1976;**2**:203-9.
22. Edwards DI. The action of metronidazole on DNA. *J Antimicrob Chemother* 1977;**3**:43-8.
23. Cone R, Hasan SK, Lown JW, Morgan AR. The mechanism of degradation of DNA by streptonigrin. *Can J Biochem* 1976;**54**:219-23.
24. Brabek, V, Palecek EC. Interaction of nucleic acids with electrically charged surfaces. III Surface denaturation of DNA on the mercury electrode in two potential regions. *Stud Biophys* 1976;**60**:105-10.
25. Nurnberg HW, Valenta P. Bioelectrical behaviour and deconformation of native DNA at charged interfaces. *Colston Papers* 1978;**29**:201-29.
26. Edwards DI, Knight RC, Kantor I. Interaction of nitroimidazole drugs with DNA. In: Siegenthaler W, Luthy R, eds. *Current Chemotherapy*. Washington: American Society for Microbiology, 1978:714-6.

27. Rowley DR, Knight RC, Skolimowski IM, Edwards DI. The effect of nitroheterocyclic drugs on DNA: an in-vitro model of cytotoxicity. *Biochem Pharmacol* 1979;**28**:3009-13.
28. Knight RC, Skolimowski IM, Edwards DI. The interaction of reduced metronidazole with DNA. *Biochem Pharmacol* 1978;**27**:2089-93.
29. Knight RC, Rowley DA, Skolimowski IM, Edwards DI. Mechanism of action of nitroimidazole antimicrobial and antitumour radiosensitizing drugs: effects of reduced misonidazole on DNA. *Int J Radiat Biol* 1979;**36**:367-77.
30. Edwards DI, Rowley DA, Knox RJ, Skolimowski IM, Knight RC. The nature of DNA damage induced by electrolytically reduced nitroimidazole drugs. In: Nelson JD, Grassi C, eds. *Current Chemotherapy and Infectious Disease*. Washington: American Society for Microbiology, 1980: 561-3.
31. Breccia A, Rimondi C, Adams GE. *Radiosensitizers of Hypoxic Cells*. Amsterdam: Elsevier (North Holland), 1979.
32. Wardman P. The use of nitroaromatic compounds as hypoxic cell radiosensitizers. *Curr Top Radiat Res Quart* 1977;**11**:347-98.
33. Adams GE, Flockhart IR, Smithen CE, Stratford IJ, Wardman P, Watts ME. Electron-affinic sensitization. VII A correlation between structures, one-electron redox potentials and efficiences of nitroimidazoles as hypoxic cell radiosensitizers. *Radiat Res* 1978;**67**:9-20.
34. Brown JM, Yu NY, Cory MJ, Bicknell RB, Taylor DL. In-vivo evaluation of the radiosensitizing and cytotoxic properties of newly synthesized electron-affinic drugs. *Br J Cancer* 1978;**37**, suppl 3:206-11.
35. Mandel M, Honigberg BM. Isolation and characterization of DNA of two species of *Trichomonas* Donné. *J Protozool* 1964;**11**:114-6.
36. Gelderman AH, Keister DB, Bartgis L, Diamond LS. Characterization of the deoxyribonucleic acid of representative strains of *Entamoeba histolytica, E histolytica*-like amoeba and *E moshkovskii*. *J Parasitol* 1971;**57**:906-11.
37. Gelderman AH, Bartgis L. Keister DB, Diamond LS. A comparison of the genome size and thermal denaturation-derived base composition of DNAs from several members of *Entamoeba* (histolytica group). *J Parasitol* 1971;**57**:912-6.
38. Reeves RE, Lushbaugh B, Montalbo FE. Characterization of deoxyribonucleic acid of *Entamoeba histolytica* by caesium chloride density centrifugation. *J Parasitol* 1971;**57**:939-44.
39. Meingassner JG, Mieth H. Cross-resistence of trichomonads to 5-nitroimidazole derivatives. *Experientia* 1976;**32**:183-4.
40. Meingassner JG, Mieth H, Czok R, Lindmark DG, Muller M. Assay conditions and the demonstration of nitroimidazole resistance in *Tritrichomonas foetus*. *Antimicrob Agents Chemother* 1978;**13**:1-3.
41. Meingassner JG, Thurner J. Strain of *Trichomonas vaginalis* resistant to metronidazole and other 5-nitroimidazoles. *Antimicrob Agents Chemother* 1979;**15**:254-7.
42. Mack S, Muller M. Effect of oxygen and carbon dioxide on the growth of *Trichomonas vaginalis* and *Tritrichomonas foetus*. *J Parasitol* 1978;**64**:927-9.
43. Ralph ED, Clarke DA, Inactivation of metronidazole by anaerobic and aerobic bacteria. *Antimicrob Agents Chemother* 1978;**14**:377-83.
44. Edwards DI, Thompson EJ, Tomusange J, Shanson D. Inactivation of metronidazole by aerobic organisms. *J Antimicrob Chemother* 1979;**5**:315-6.
45. McCalla DR. Biological effects of nitrofurans. *J Antimicrob Chemother* 1977;**3**:517-20.
46. McCalla DR. Nitrofurans. In: Hahn O, ed. *Antibiotics*. Vol 5. New York: Springer-Verlag, 1980: 176-213.
47. Britz ML, Wilkinson RG. Isolation and properties of metronidazole-resistant mutant of *Bacteroides fragilis*. *Antimicrob Agents Chemother* 1979;**16**:19-27.
48. Ingham HR, Eaton S, Venables CW, Adams PC. *Bacteroides fragilis* resistant to metronidazole after long-term therapy. *Lancet* 1978;**i**:214.

Br J Vener Dis 1980;56:291-6.

Quantitative microhaemagglutination assay for Treponema pallidum antibodies in experimental syphilis

ROBERT R TIGHT AND ARTHUR C WHITE

From the Division of Infectious Diseases, Department of Medicine, Indiana University School of Medicine, Indianapolis, USA

SUMMARY The quantitative microhaemagglutination assay for *Treponema pallidum* antibodies (MHA-TP) was studied in 52 untreated and treated rabbits with experimental syphilis. Rabbits with incubating experimental syphilis were cured or inadequately treated with penicillin G and some cured rabbits were later reinfected. MHA-TP conversion occurred within 45 days in untreated rabbits. Titres reached peak levels about four months after inoculation and remained relatively high for up to two years. The quantitative MHA-TP test differentiated between rabbits cured of experimental incubating syphilis and those untreated and inadequately treated. MHA-TP titres decreased after treatment given six or 12 months after inoculation but reversion did not occur. MHA-TP conversion or significant increases in titre occurred as soon as seven days after reinfection and preceded corresponding changes in a quantitative non-treponemal test. The MHA-TP is useful as a screening test for treponemal antibodies in rabbits. The quantitative MHA-TP in humans after treatment for syphilis and reinfection deserves further study.

Introduction

The microhaemagglutination assay for *Treponema pallidum* antibodies (MHA-TP) is a relatively new treponemal serological test for syphilis. Its present role was recently reviewed.[1] Compared with other treponemal tests, such as the fluorescent treponemal antibody absorption (FTA-ABS) or *Treponema pallidum* immobilisation (TPI) tests, it has certain advantages. It uses a sensitive technique for antibody detection, passive haemagglutination. Microvolume methods require less reagent and less material. Results can be evaluated without microscopy and are easier to interpret.[1] The MHA-TP test is less expensive than other treponemal tests, is relatively reliable and reproducible,[2] and can be automated.[1-4] Test materials are available in kit form, making the testing of numerous specimens practical. It may combine the simplicity and lower cost of non-treponemal tests with the sensitivity and specificity of treponemal tests. It is potentially useful both as a screening and as a confirmatory test for use in conjunction with non-treponemal tests.[1]

A few studies of the MHA-TP in animals with experimental treponemal infections have been reported. It appears to be more useful for screening rabbits for possible *Treponema cuniculi* infections than the Venereal Disease Research Laboratory (VDRL) test and can be useful in quantitating antibodies to pathogenic treponemes.[3 5 6] Experience with other quantitative treponemal tests is limited, owing partly to the relative difficulty in performing them.[7 8]

In the present study we attempted to clarify the meaning and interpretation of the quantitative MHA-TP in rabbits with experimental *T pallidum* infections. We determined the range and duration of MHA-TP titres (a) in untreated rabbits; (b) in rabbits treated at varying intervals after infection; and (c) in rabbits cured of experimental incubating syphilis (EIS) which were then reinfected. For comparison, a quantitative VDRL test was also performed on all specimens.

Materials and methods

EXPERIMENTAL INFECTIONS

T pallidum, Nichols strain, was originally obtained from the Center for Disease Control, Atlanta, Georgia, and was maintained by serial passage in

Address for reprints: Dr R R Tight, c/o VA Hospital, North Elm and 21st Avenue, Fargo, North Dakota 58102, USA

Received for publication 31 December 1979

rabbit testes or by freezing at −70°C. Male New Zealand white rabbits were obtained locally. Suspensions of freshly thawed *T pallidum* were counted by the method of Morgan and Vryonis[9] and diluted appropriately, so that approximately 5×10^4 organisms in 0·5 ml total volume were injected intratesticularly into one testis of each infected rabbit. During each inoculation session at least two rabbits were left untreated as controls for infectivity of the suspension. After inoculation, rabbits were examined regularly for two months or until palpable testicular induration was detected, at which time testicular material was aspirated for darkfield examination. Rabbits were considered to be infected if testicular induration developed, aspirated testicular tissue was darkfield-positive, and MHA-TP seroconversion occurred. Those rabbits treated within 14 days after infection which failed to develop orchitis (testicular induration and darkfield-positive testicular tissue aspirate) or a reactive MHA-TP were considered to have been infected if the untreated control animals were infected.

THERAPY

Certain rabbits were treated with single doses of benzathine penicillin G (BPG) or aqueous crystalline penicillin G (ACPG) 100 000 units per kg intramuscularly. BPG doses were comparable to those used in the treatment of human syphilis[10] and were presumed to cure the experimental infections because the serum penicillin activity lasted eight or more days (R Tight, unpublished observations). This is longer than the four-day duration of activity previously shown to eradicate experimental early syphilis uniformly.[11] With ACPG therapy, very high serum penicillin concentrations are obtained, but they are of such short duration that single-dose therapy is "inadequate" for the uniform eradication of *T pallidum* infections.[12]

TREATMENT GROUPS

Four groups of rabbits were studied. One group of 16 rabbits remained untreated. A second group received therapy during the incubating stage of infection—that is, within the first 14 days after inoculation; of these, 10 rabbits received a single dose of BPG on day 7, and 10 on the fourteenth day, after inoculation and nine were given a single dose of ACPG on day 7. A third group of two rabbits was treated with a single dose of BPG on day 180 after inoculation. The fourth group of five rabbits was treated one year after inoculation with weekly doses of BPG for three weeks to approximate the duration of treatment of human syphilis of more than one year's duration.[10]

REINFECTION

Twelve rabbits cured of EIS were reinfected, 4-10 months after the initial inoculation, with 5×10^4 *T pallidum* intratesticularly in the opposite testis from that used initially. After reinfection, rabbits were examined regularly until orchitis developed.

SEROLOGICAL TESTS

Serum samples were obtained from each rabbit as follows: before infection; 7, 14, 30, 45, and 60 days after infection; then every two months. Post-reinfection sera were obtained on days 7, 14, 21, and 30. MHA-TP and VDRL slide tests were performed on all sera. The procedures recommended by the manufacturer (Sera-Tek Treponemal Antibody Test, manufactured by Fujizoki Pharmaceutical Co Ltd, Tokyo, Japan, and distributed by Ames Co, Elkhart, Indiana) and the Center for Disease Control, Atlanta, Georgia, were followed in performing the MHA-TP.[13]

The result for any specimen with 1+ or greater haemagglutination was interpreted as reactive. Specimens giving − or ± results were read as non-reactive. Each specimen reactive at a 1/80 dilution was titrated by testing serial two-fold dilutions of the absorbed serum (1/20) in absorbing diluent; the titre was read as the last serum dilution giving a reactive result. The VDRL slide test was performed as recommended by the Center for Disease Control.[14]

Results

UNTREATED RABBITS

All 16 untreated rabbits were followed for 12 months after infection; of these, seven were followed for a total of 24 months. Orchitis developed in all 16 untreated rabbits 15 to 50 days (mean, 24 days) after inoculation.

Serial MHA-TP and VDRL titres in untreated rabbits are shown in fig 1. VDRL titres appeared to rise slightly faster and reach peak levels slightly earlier after inoculation than MHA-TP titres, as indicated by the slopes of the lines connecting median titres between days 14 and 60. VDRL titres then decreased fairly rapidly even without treatment, as has been known.[15] Our results confirm previous reports of both false-positive and false-negative VDRL test results in rabbits.[15-17] Thus, three of 16 rabbits had reactive VDRL tests at low titres (up to 1/2) before inoculation. Although rigorous attempts to exclude inapparent *T cuniculi* infections were not carried out, negative preinoculation MHA-TP test results in the rabbits in this and previous studies[17] suggest that apparently normal rabbits, with no evidence of treponemal infection, may show low-grade reactivity in the VDRL test. Furthermore, one

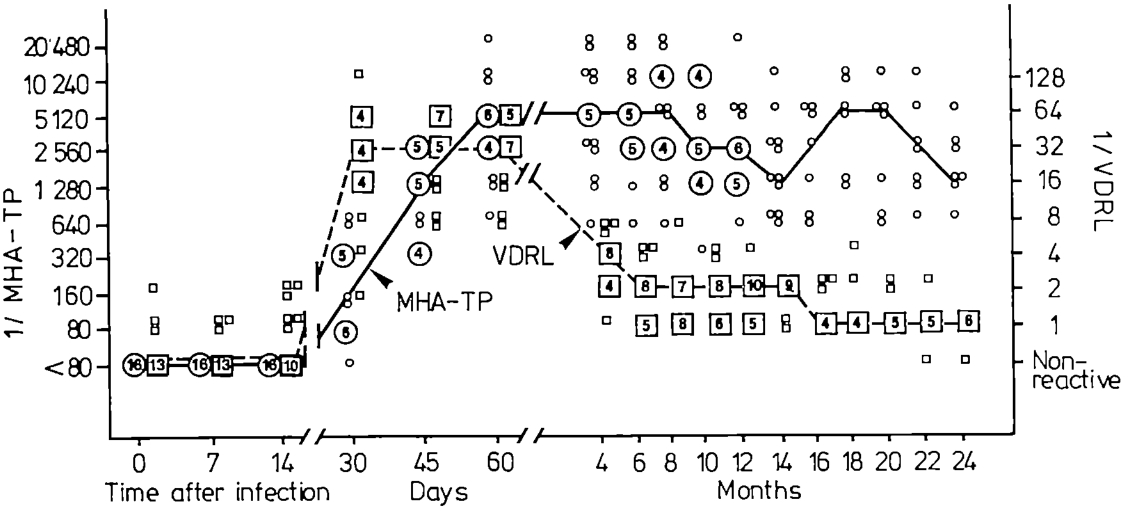


FIG 1 *Range and median MHA-TP and VDRL test reciprocal titres in sera from rabbits infected with T pallidum. Individual rabbit MHA-TP (○) and VDRL (□) reciprocal titres are shown for each time a blood sample was taken. Larger symbols indicate four or more identical results. Lines connect median reciprocal titres.*

of seven rabbits had become VDRL-negative by 22 months after inoculation without treatment, confirming that treponemal tests may be necessary to detect asymptomatic treponemal infection.[15 16]

MHA-TP results on all sera before inoculation and on day 7 and on day 14 after inoculation were non-reactive. By day 30 however 15 of 16 sera were reactive, and by day 45 all were reactive. After conversion, MHA-TP titres remained relatively high for up to 24 months. Cox *et al*[3] reported similar results. The considerable variability in titres between rabbits is illustrated in fig 1 and has been reported by others in human and experimental syphilis.[2-4 18]

No untreated rabbit had detectable orchitis or a reactive MHA-TP result within the initial 14 days after inoculation. Thus, EIS in this study is similar to EIS as studied by others[19 20] and to incubating human syphilis.[21]

RABBITS TREATED FOR EIS

Twenty rabbits with EIS were treated with BPG on day 7 or day 14 after inoculation (table). None subsequently developed orchitis. Treatment on day 7 prevented MHA-TP conversion in all rabbits. Delaying treatment until day 14 resulted in most rabbits (seven of 10) developing reactive MHA-TP results at low (compared with untreated animals) titres (table) and slightly later after inoculation. Thus, MHA-TP conversion occurred on day 30 in 15 of 16 untreated rabbits but in only three of 10 rabbits treated on day 14 (P<0·01 using the "fourfold" table method of calculating χ^2 with Yates's correction[22]). Peak MHA-TP titres were $\leqslant$1/320 in nine of 10 rabbits treated on day 14 whereas peak titres $\geqslant$1/1280 occurred in all 16 untreated rabbits (P<0·001). Three rabbits treated on day 14 remained MHA-TP-negative for at least six months of observation, two reverted to non-reactive at six months, and the remainder were reactive at constantly low or, decreasing, titres ($\leqslant$1/640) for up to 12 months of observation. The MHA-TP was thus useful in differentiating between rabbits cured of EIS (especially those treated on day 7) and untreated or inadequately treated rabbits.

Nine rabbits with EIS were treated on day 7 after

TABLE *Peak MHA-TP titres and interval after inoculation in untreated rabbits infected with T pallidum and in those treated 7 and 14 days after infection with 100 000 units benzathine penicillin G per kg*

		Reciprocal peak (MHA-TP) titre		*Time (days) after inoculation*	
Rabbit group	*No*	*Median*	*Range*	*Median*	*Range*
Untreated	16	10 240	1280-20 480	120	60-360
Treated (day 7)	10	<80	<80		
Treated (day 14)	10	160	<80-640	60*	30-120*

*Values for those seven rabbits developing reactive MHA-TP results

LIBRARY

inoculation with the inadequate ACPG regimen. Four developed orchitis and high MHA-TP titres indicative of treatment failure. Onset of orchitis (33-50 days after inoculation; mean, 45 days) and seroconversion (on day 45 for all four) were slightly delayed compared with untreated rabbits. Five of the rabbits were apparently cured with the inadequate regimen. Peak titres of rabbits not cured with the ACPG regimen (>1/5120 at two months) were similar to peak titres in untreated animals.

RABBITS TREATED SIX OR 12 MONTHS AFTER INFECTION

Rabbits treated with BPG at six or 12 months were followed for 24 months after inoculation. MHA-TP titres of treated and untreated rabbits were similar up to the time of treatment (fig 2). After treatment MHA-TP titres decreased. The two rabbits treated at six months had 16-fold and 32-fold decreases in titre in the subsequent six months and a further fourfold decrease between 12 and 24 months. Five rabbits treated at 12 months subsequently had eight-fold to 32-fold decreases in MHA-TP titre. Such decreases were greater than the smaller fluctuations in titres that occurred in the seven untreated rabbits followed for 24 months. By 22 months titres in treated rabbits were significantly lower than those in the untreated animals (fig 2). Thus, although some overlap remained, six of seven rabbits treated at six or 12 months had MHA-TP titres <1/320 whereas six of seven untreated rabbits had MHA-TP titres >1/1280 ($P<0.05$).

REINFECTION AFTER CURE OF EIS

Results of serial post-reinfection MHA-TP and VDRL tests show some differences compared with initial infections. MHA-TP conversion occurred sooner after reinfection than after initial infection. Six of nine reinfected seronegative rabbits had reactive serum MHA-TP results seven days after reinfection whereas none of 16 specimens on day 7 was reactive after initial infections ($P<0.01$). Similarly, significant (fourfold and eightfold) rises in titre had occurred by day 7 after reinfection in two of three animals whose MHA-TP results before reinfection were still reactive (1/80 for all three). Relatively high MHA-TP titres also appeared to occur sooner after reinfection than after initial infection. Two rabbits had titres of 1/640 by day 7 after reinfection and another three had titres >1/5120 by day 30 after reinfection, which were higher than comparable titres after initial infections (fig 1).

After initial infections, serological conversion or significant rise in titre occurred with approximately the same rapidity (that is, usually between day 14 and day 30) with both serological tests. After reinfection, however, conversion or significant rise in titre

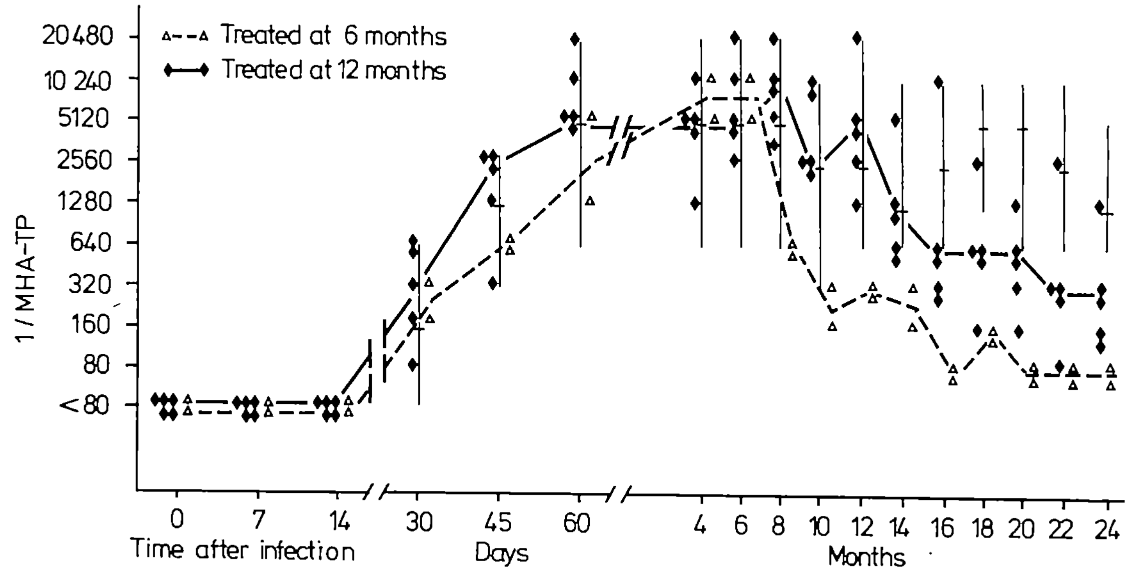


FIG 2 *Range and median reciprocal MHA-TP titres in sera from two rabbits treated six months after infection and in five rabbits treated 12 months after infection. Vertical lines with short intersecting horizontal lines indicate ranges and medians of reciprocal titres in untreated rabbits (from fig 1). Other lines connect median reciprocal titres for each treatment group.*

occurred by day 7 in eight of 12 reinfected rabbits with the MHA-TP test but in none with the VDRL test ($P<0 \cdot 01$). Overall, MHA-TP conversion or significant rise in titre preceded corresponding VDRL changes in nine of 12 rabbits and occurred simultaneously on day 30 in the other three. Eight reinfected rabbits developed orchitis 19 to 32 days (mean, 25 days) after reinoculation. The other four were killed between day 14 and 40 after reinfection, possibly before orchitis could develop.

Discussion

Results confirmed that the MHA-TP is useful for screening rabbits to be used for studies of experimental syphilis.[3 6] The VDRL slide test may give reactive results in up to 40% of apparently normal rabbits.[17] False-positive MHA-TP results in rabbits not infected with pathogenic treponemes have not, to our knowledge, been reported, although three chimpanzees experimentally infected with *T pallidum* had pre-existing MHA-TP titres of 1/320 to 1/5120, and reactive FTA-ABS test results of unknown origin.[3] Conversely, nine of nine rabbits infected with *T pertenue,* 10 of 10 infected with *T cuniculi,* and 54 of 55 infected with *T pallidum* had reactive MHA-TP results.[3] The specimen giving the one non-reactive result may have been taken on day 20 after inoculation, before MHA-TP conversion occurred.

The FTA-ABS test has been the most satisfactory serological test in experimental *T pallidum* infections in rabbits, with a specificity approaching 100%, greater sensitivity than the TPI test, and greater sensitivity and specificity than the VDRL slide test.[15 16] However, performance of the FTA-ABS test on rabbit sera requires the use of fluorescein-labelled antirabbit globulin. In one study, two to three absorptions of the globulin with liver or bone marrow powder were sometimes necessary to remove non-specific staining.[16] Thus, the MHA-TP test on rabbit sera seems simpler to perform than the FTA-ABS test. Comparative studies of the two tests in experimental syphilis would be useful. If the specificity and sensitivity of the two tests were similar, the technical simplicity of the MHA-TP test could make it preferable for screening rabbits for pre-existing treponemal antibodies in studies of experimental syphilis.

In this study, the quantitative MHA-TP test was useful in differentiating curative treatment of EIS from untreated or inadequately treated EIS. Treatment of EIS on day 7 completely prevented orchitis and seroconversion. In contrast, untreated rabbits, and rabbits not cured with the inadequate ACPG regimen, developed orchitis and high MHA-TP titres. Results of this study suggest that rabbits with EIS could be treated on day 7 with drug regimens of similar duration to those used in the treatment of gonorrhoea in humans. Effective regimens would uniformly prevent orchitis and seroconversion. Similar animal studies[19] led to human studies[21] that established the effectiveness of the current aqueous procaine penicillin G (APPG) (4 800 000 units) treatment regimen for gonorrhoea in eradicating human incubating syphilis. In contrast, spectinomycin failed to eradicate EIS,[20] which suggests it would also be ineffective in humans. If tetracycline, ampicillin, and amoxycillin were effective in such studies, the results would support the recent suggestion that patients with incubating syphilis are "likely to be cured" by recommended schedules for the treatment of gonorrhoea with these drugs.[23] Newer drugs for gonorrhoea, such as cefoxitin and cefuroxime, could be similarly studied. Groups of animals large enough to detect regimens which are inadequate in curing all infections, yet capable of eradicating some, would be needed. The ACPG regimen in this study was an example of such an inadequate regimen, and similar ACPG therapy failed to cure a case of incubating human syphilis.[12] Similarly, APPG (600 000 units), tetracycline (3 g), and chloramphenicol regimens eradicated incubating syphilis in some, but not all, patients treated.[21]

Although the MHA-TP did not become non-reactive when treatment was first given six or 12 months after infection (like the FTA-ABS test after treatment of human syphilis), treatment was followed by significantly decreasing titres. Results of reinfection of rabbits cured of EIS suggested that the quantitative MHA-TP was an earlier indicator of reinfection than the quantitative VDRL slide test. We are unaware of any other studies of the quantitative MHA-TP after therapy or reinfection in experimental syphilis, although Collart *et al*[7] showed that the quantitative TPI test titre fell more rapidly after treatment two years after inoculation than in untreated controls. Whether or not similar results occur in humans is unclear. Logan and Cox reported significant decreases in MHA-TP titres in 11 of 21 patients with early syphilis one year after therapy,[4] whereas O'Neill reported slowly decreasing MHA-TP titres after treatment.[24]

In general, important shortcomings of studies of the quantitative MHA-TP in humans have been the small numbers of patients and relatively short follow up.[1 2 4 24] Similarly, it is not known if the MHA-TP would be a more sensitive indicator of reinfection than a non-treponemal test in humans, although in one case it was.[25] Such data from human studies would clearly be of interest and could readily be obtained by performing sequential quantitative non-treponemal and MHA-TP tests on follow-up sera from patients treated for syphilis. Even if MHA-TP titres decreased in humans after effective treatment, this might not be of any practical importance, since quantitative non-treponemal tests are satisfactory for post-treatment follow up. However, the possibility that the quantitative MHA-TP could be an earlier indicator of reinfection has potential clinical importance, since it might enable earlier diagnosis, prompter therapy, and interruption of spread of infectious syphilis. Thus, further studies of the quantitative MHA-TP in humans after treatment for syphilis and reinfection would be worthwhile.

This work was supported in part by a grant-in-aid from the Attorney General of Indiana Public Health Trust. We thank Phillip Sylbert (deceased), Ames Co, Elkhart, Indiana, for supplying kits (Sera-Tek Syphilis Serodiagnosis Kits) used for performing MHA-TP tests. We also thank James Teipen for excellent technical assistance and Dorothy Jones for typing the manuscript.

References

1. Rudolph AH. The microhemagglutination assay for *Treponema pallidum* antibodies (MHA-TP), a new treponemal test for syphilis: where does it fit? *J Am Vener Dis Assoc* 1976;**3:**3-8.
2. Coffey EM, Bradford LL, Naritomi LS, Wood RM. Evaluation of the qualitative and automated quantitative microhemagglutination assay for antibodies to *Treponema pallidum. Appl Microbiol* 1972;**24:**26-30.
3. Cox PM, Logan LC, Norins LC. Automated, quantitative microhemagglutination assay for *Treponema pallidum* antibodies. *Appl Microbiol* 1969;**18:**485-9.
4. Logan LC, Cox PM. Evaluation of a quantitative automated microhemagglutination assay for antibodies to *Treponema pallidum. Am J Clin Pathol* 1970;**53:**163-6.
5. Okamoto S, Yoshitsugu T. Studies on *Treponema pallidum* haemagglutination antibodies. I TPHA antibodies in experimental syphilitic rabbits. *Br J Vener Dis* 1971;**47:**77-80.
6. Tight RR, Perkins RL. *Treponema pallidum* infection in subcutaneous polyethylene chambers in rabbits. *Infect Immun* 1976;**13:**1606-12.
7. Collart P, Franceschini P, Durel P. Experimental rabbit syphilis. *Br J Vener Dis* 1971;**47:**389-400.
8. Turner TB, Hollander DH. Biology of the treponematoses. *Monograph Series No 35,* Geneva: World Health Organisation, 1957:158-66.
9. Morgan HJ, Vryonis GP. A method for the quantitation of inocula in experimental syphilis. *Am J Syph Gonorrhea Vener Dis* 1938;**22:**462-9.
10. US Department of Health, Education, and Welfare. Syphilis, CDC recommended treatment schedules. *Morbidity Mortality Weekly Rep* 1976;**25:**101-7.
11. Arnold RC, Mahoney JF, Cutler JC. Reinfection in experimental syphilis in rabbits following penicillin therapy. II Reinfection in early latent syphilis. *Am J Syph Gonorrhea Vener Dis* 1947;**31:**489-92.
12. Wright JT. Single-dose penicillin therapy. *Br J Vener Dis* 1975;**51:**410.
13. US Department of Health, Education and Welfare. Microhemagglutination assay for *Treponema pallidum* antibodies (MHA-TP); provisional technique. Atlanta GA: Center for Disease Control, 1977.
14. US Department of Health, Education and Welfare. *Manual of Tests for Syphilis.* Washington DC: US Government Printing Office, 1969:33-9.
15. Miller JM. *Spirochetes in Body Fluids and Tissues.* Springfield, Illinois: Charles C Thomas, 1971:3-58.
16. Mothershed SM, Yobs AR, Clark JW. Comparison of the VDRL slide, TPI, and FTA-ABS tests in experimental syphilis in rabbits. *Br J Vener Dis* 1967;**43:**267-71.
17. Pannu JS, Rosenberg MA, Israel CW, Smith JL. Incidence of reactive VDRL tests in the normal rabbit. *Br J Vener Dis* 1967;**43:**114-6.
18. O'Neill P, Warner W, Nicol CS. *Treponema pallidum* haemagglutination assay in the routine serodiagnosis of treponemal disease. *Br J Vener Dis* 1973;**49:**427-31.
19. Lucas JB. The effects of gonorrhea therapy on incubating syphilis. In: Nicholas L, ed. *Sexually Transmitted Diseases.* Springfield, Illinois: Charles C Thomas, 1973:71-83.
20. Petzoldt D. Effect of spectinomycin on *T pallidum* in incubating experimental syphilis. *Br J Vener Dis* 1975;**51:**305-6.
21. Schroeter AL, Turner RH, Lucas JB, Brown WJ. Therapy for incubating syphilis, effectiveness of gonorrhea treatment. *JAMA* 1971;**218:**711-3.
22. Hill AB. *Principles of Medical Statistics.* 9th ed. New York: Oxford University Press, 1971:172-4.
23. US Department of Health, Education, and Welfare. Gonorrhea, CDC recommended treatment schedules. *Morbidity Mortality Weekly Rep* 1979;**28:**13-21.
24. O'Neill P. A new look at the serology of treponemal disease. *Br J Vener Dis* 1976;**52:**296-9.
25. Jephcott AE, Beveridge MAM, Sequeira PJL. Early TPHA response in primary syphilis. *Br J Vener Dis* 1977;**53:**147.

Br J Vener Dis 1980;**56**:297-301.

Autolymphocytotoxins in syphilis

N H L DE JONG, J A M KOEHORST, J J VAN DER SLUIS, AND A M BOER
From the Department of Dermatovenereology, Erasmus University, Rotterdam, the Netherlands

SUMMARY Nineteen of 23 serum samples from patients with secondary syphilis, six of 24 from patients with primary syphilis, and four of seven from patients with early latent syphilis had complement-dependent autolymphocytotoxic reactivity at 15°C. After treatment, the reactivity was decreased. It was not directed only against autologous lymphocytes but also against allogeneic lymphocytes. Sephadex G-200 gel filtration showed that the lymphocytotoxins were present in the IgM-containing exclusion peak. Furthermore, serum samples that had high antibody titres in the Reiter protein complement-fixation test or Wassermann-Kolmer reaction also had a high frequency of cold-reacting autolymphocytotoxins. This indicates that the autolymphocytotoxic reactivity is related to the humoral response in syphilis.

Introduction

In patients with early syphilis there is an extensive response by the antibody-synthesising immune system. It is known that a hyperimmunoglobulinaemia may develop,[1 2] and antibodies of several specificities are produced. However the humoral immune response does not appear to protect the human host against progression of the disease. This has led to the investigation of the role of cellular mechanisms in the immune response to *Treponema pallidum*.

The results of such investigations may point to a disturbance of T-lymphocyte functions in patients with early syphilis. It has been found (a) that patients with early syphilis usually do not show a delayed hypersensitivity reaction to *T pallidum* antigens;[3-5] (b) that a depletion of lymphocytes in the thymus-dependent areas of lymph nodes from patients with early syphilis may occur;[6] and (c) that lymphocytes from patients with primary or secondary syphilis cultured in the presence of autologous plasma show a reduced response to phytohaemagglutinin (PHA).[7]

The investigations of Levene *et al*[7] and Kantor[8] have shown that plasma from patients with secondary syphilis also causes a reduction in the PHA-response of normal lymphocytes. It is suggested that plasma from patients with secondary syphilis contains an inhibiting factor which may be a treponemal product or an autoantibody directed against lymphocytes. A defect in the T-cell population might be related to the presence of this inhibiting factor.[7] Naito *et al*[9] have found that sera from patients with syphilis exert a cytotoxic effect on normal lymphocytes. We have found that sera from patients with early syphilis contain a lymphocytotoxin directed against the patient's own lymphocytes and have investigated some of its properties.

Patients and methods

STUDY POPULATION

Fifty-four patients with untreated early syphilis (of less than two years' duration) were studied. The diagnosis was confirmed by darkfield demonstration of *T pallidum* or by a positive result to the *T pallidum* immobilisation (TPI) test or by both. Twenty-four of the patients had primary, 23 secondary, and seven early latent syphilis. The mean age of the patients was 29 years, with a range of 15-54 years. The sex ratio (male-to-female) was 3·5:1.

Treatment consisted of three intramuscular injections of procaine benzylpenicillin 600 000 units in oil suspension plus 2% aluminium monostearate (Almopen) a week for six weeks.

The TPI and Reiter protein complement-fixation (RPCF) tests and the Kolmer modification of the Wassermann reaction were carried out at the National Institute of Public Health, Bilthoven, the Netherlands. Serum and blood samples for the preparation of cell suspensions were obtained from 42 patients just before treatment. Serum samples were obtained from the other 12 patients before and at the end of treatment. In the latter cases, the initial and final samples were tested simultaneously by the

Address for reprints: Dr N H L de Jong, Research Laboratory, Department of Dermatovenereology, Erasmus University, Dr Molewaterplein 50, Postbus 1738, Rotterdam, the Netherlands

Received for publication 11 July 1979

microlymphocytotoxic test against lymphocytes isolated from blood specimens obtained at the end of the treatment period. The pretreatment serum samples were stored at −70°C.

CONTROLS

Serum samples and cell suspensions obtained from 18 healthy subjects were used as controls. The mean age of the controls was 29 years with a range of 18-45 years. The sex ratio (male-to-female) was 2·6:1.

PREPARATIONS OF LYMPHOCYTES

Mononuclear cells were isolated by the method of Bøyum[10] with minor modifications. Peripheral blood anticoagulated with 20 IU preservative-free heparin per ml (Thromboliquine, Organon, Oss, the Netherlands) was diluted two-fold with Dulbecco's phosphate-buffered saline without Ca^{++} and Mg^{++}, pH 7·3 (PBS). Three millilitres of the diluted blood was layered on to 1·5 ml Ficoll-Isopaque (Lymphoprep, Nyegaard & Co, Oslo, Norway) in glass tubes with an inner diameter of 9 mm. After centrifugation at 800 × *g* for 15 minutes at room temperature, the cells at the interface were harvested and washed three times with PBS-BSA (PBS supplemented with 1% w/v demineralised bovine serum albumin; Organon, Oss, the Netherlands). After the final wash, the cells were resuspended in MEM (Eagle's minimum essential medium spinner-modified; Difco Laboratories, Detroit, Michigan, USA) at a concentration of 1×10^6 cells/ml.

DETECTION OF AUTOLYMPHOCYTOTOXIC REACTIVITY

The autolymphocytotoxic reactivity of the sera was determined by the microlymphocytotoxic test described by Mittal *et al.*[11] The incubation time and temperature were modified according to Naito *et al*[9]. Briefly, 1 μl of the lymphocyte suspension was incubated at 15°C with the test serum under oil in disposable microtest plates (Terasaki plates, Falcon Plastics). After 30 minutes, 5 μl of rabbit complement were added and the incubation was continued for another three hours at 15°C. After incubation, 3 μl of eosin Y dye and 8 μl of 40% formalin were added sequentially to each well. With an inverted phase-contrast microscope, the degree of cytotoxicity was assessed by estimating the proportion of stained cells and scored as follows: −, 0-4% stained; ±, 5-14% stained; +, 15-24% stained; + +, 25-39% stained; + + +, 40-79% stained; and + + + +, 80-100% stained.

Investigation of the sera was carried out with the following quantities and dilutions: 1 μl and 3 μl of the undiluted serum and 1 μl of the serum diluted 1/2, 1/4, 1/8, and 1/16 in MEM.

Undiluted unabsorbed rabbit serum was used as the complement source; this was selected for good complement activity and minimal cytotoxic activity under the conditions of the test. To determine whether complement was required for the cytotoxic reaction and to exclude non-specific cytotoxic effects, each serum sample (1 μl undiluted and 1 μl diluted 1/2) was also tested with heat-inactivated complement. In each test, a positive and a negative control sample was included.

SERUM FRACTIONATION

Cytotoxic sera were fractionated by applying 5 ml of serum to a Sephadex G-200 column (2·6×80 cm). The proteins were eluted with 0·1 mol/l Tris-HCl, 0·5 mol/l NaCl buffer, pH 8·6, at a flow rate of 15 ml/hr. The protein content of the eluates was monitored by measuring the absorbance at 280 nm. Three peaks were obtained. The fractions of the first (19S) peak and the second (7S) peak were pooled and concentrated to the initial volume of the serum sample by ultrafiltration using an Amicon PM-10 membrane. The immunoglobulin composition of the peaks was checked by Ouchterlony double-diffusion using anti-IgM and anti-IgG (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam). In the first peak, which was positive for IgM, no IgG could be detected whereas in the second peak, which was positive for IgG, no IgM could be detected.

The χ^2 test with Yates's correction was used to determine whether a significant association existed between high RPCF (>1/32) or high Wassermann-Kolmer (>1/64) antibody titres and a positive autolymphocytotoxic reaction.

Results

COLD-REACTING AUTOLYMPHOCYTOTOXINS

Frequency

Pretreatment serum samples from 54 patients with early syphilis and samples from 18 controls were tested for the presence of cytotoxic reactivity against autologous lymphocytes in the microlymphocytotoxic test. Some examples of the reactivity of syphilitic sera at different serum dilutions are given in table I. In most of the reactive samples, maximal autolymphocytotoxic reactivity was found with 1 μl of undiluted serum. It rarely exceeded a titre of 1/4. When 3 μl of undiluted serum was used, a prozone effect was present with all of the reactive sera tested.

The frequency distribution of the cold autolymphocytotoxic reactivity of the sera in relation to the different stages of the disease is given in table II. Each serum sample is denoted by its maximum score

TABLE I *Example of the cold-complement-dependent autolymphocytotoxic reactivity of pretreatment sera from syphilitic patients at different serum dilutions*

		Serum amount and dilution					
Patient No	*Stage of syphilis*	*3 µl 1/1*	*1 µl 1/1*	*1 µl 1/2*	*1 µl 1/4*	*1 µl 1/8*	*1 µl 1/16*
1	secondary	±	+	±	±	−	−
2	secondary	±	+ +	+	±	±	−
3	secondary	−	+	+ +	±	±	−
4	early latent	±	+ + +	+ +	±	−	−
5	secondary	±	+ + +	+ + +	+ + +	+	±

−, 0-4% stained cells; ± 5-14% stained cells; +, 15-24% stained cells; + +, 25-39% stained cells; + + +, 40-79% stained cells; + + + +, 80-100% stained cells

TABLE II *Frequency of cold-complement-dependent autolymphocytotoxic reactivity in pretreatment sera from patients with early syphilis*

		*Cytotoxic score**					
Stage of disease	*No of patients tested*	−	±	+	+ +	+ + +	+ + + +
Primary syphilis	24	12	6 (1/1, 1/1, 1/1, 1/1, 1/2, 1/2)	0	4 (1/1, 1/1, 1/1, 1/1)	1 (1/1)	1 (1/2)
Secondary syphilis	23	0	4 (1/1, 1/1, 1/2, 1/8)	4 (1/1, 1/1, 1/1, 1/2)	8 (1/1, 1/1, 1/1, 1/1, 1/1, 1/2, 1/2, 1/2)	6 (1/1, 1/1, 1/1, 1/1, 1/2, 1/4)	1 (1/1)
Early latent syphilis	7	3	0	1 (1/4)	2 (1/1, 1/2)	1 (1/1)	0
Controls	18	16	1 (1/4)	1 (1/1)	0	0	0

*Highest serum dilution showing this cytotoxic score given in brackets
−, ±, +, + +, + + +, + + + + see table I

and the highest serum dilution showing this score. The highest incidence of autolymphocytotoxins occurred in the sera of patients with secondary syphilis. Positive reactions were present in 19 (83%) of 23 of the patients. Not only were a larger number of samples reactive at this stage of the disease but the reactivity of sera from these patients against their own lymphocytes was also stronger than the autolymphocytotoxic reactivity of the sera from the patients at the other stages of the disease. Twenty-five per cent of the sera from patients with primary syphilis also showed an autolymphocytotoxic reaction. Four of seven sera from patients with early latent syphilis were cytotoxic whereas the sera from controls were inactive, except for one which showed a + reaction. When tested with heat-inactivated rabbit complement none of the reactive sera was positive.

Properties

Three of the pretreatment serum samples were tested both at 15°C and at 37°C. When the test was performed at 15°C, autolymphocytotoxic reactivity was present in two of these samples, both showing a + + reaction. No reactivity was found when the test was carried out at 37°C.

Pretreatment serum samples from eight syphilitic patients containing cytotoxic reactivity against autologous lymphocytes were also tested against allogeneic lymphocytes. All of these samples also gave positive reactions against allogeneic cells.

The results obtained in the cytotoxic test for three pretreatment sera fractionated by elution from Sephadex G-200 are given in table III. In two cases, whole serum and the serum fractions were tested against autologous lymphocytes. Whole serum and fractions of the third patient were tested against allogeneic lymphocytes obtained from a healthy donor, since the patient was no longer available for study. The gel filtration experiments showed that the lymphocytotoxic reactivity was located in the IgM-containing exclusion-peak.

Heating the sera and the IgM-containing peak obtained by gel filtration for 30 minutes at 56°C caused a decrease in cytotoxic reactivity.

EFFECT OF TREATMENT

Serum samples obtained from patients at the end of treatment and before treatment were tested simultaneously against autologous lymphocytes isolated from peripheral blood obtained at the end of treatment. The post-treatment samples had a lower

TABLE III *Lymphocytotoxic reactivity of whole sera and Sephadex G-200 fractions*

		Serum amount and dilution					
Patient No	*Serum or eluate*	*3 μl 1/1*	*1 μl 1/1*	*1 μl 1/2*	*1 μl 1/4*	*1 μl 1/8*	*1 μl 1/16*
1*	Serum	+ +	+ + + +	+ + + +	+ + +	+	±
	19S peak	+ +	+ +	±	–	–	–
	7S peak	–	–	–	–	–	–
2*	Serum	±	+ +	+ + +	+ +	+	±
	19S peak	±	+	+	+	±	±
	7S peak	–	–	–	–	–	–
3†	Serum	+	+ + +	+ +	+	±	–
	19S peak	+	+	+	±	–	–
	7S peak	–	–	–	–	–	–

*Whole serum and fractions were tested simultaneously against autologous lymphocytes
†Whole serum and fractions were tested simultaneously against allogeneic lymphocytes obtained from a healthy donor
–, ±, +, + +, + + +, + + + + see table I

cytotoxic score than the pretreatment samples (table IV).

ASSOCIATION WITH RPCFT AND KOLMER REACTION

Most sera with titres above 1/32 in the RPCFT showed a positive reaction in the cytotoxic test (20 out of 24) whereas most sera with RPCF titres ⩽1/32 gave negative results in the cytotoxic test (21 out of 30). This association was statistically significant ($\chi_1^2 = 13 \cdot 18$; P<0·001). A similar association was present between the cytotoxic reaction and the titres in the Kolmer test. When looking at sera with titres >1/64, 22 of 31 showed a positive cytotoxic reaction. Sera with titres ⩽1/64 showed a negative reaction in 16 of 23. Here also a statistically significant association ($\chi_1^2 = 7 \cdot 17$; P<0·01) was found.

Discussion

Cold complement-dependent lymphocytotoxins (CLC) reacting optimally at 15°C are found in a great number of infectious diseases,[12-14] autoimmune diseases,[9, 15-17] after vaccination,[18] in pregnancy, and in syphilis.[9] Although in most investigations panels of allogeneic lymphocytes are used as target cells, the assumption has been made that CLC are also autocytotoxic.

This assumption has also been made for syphilis by Naito *et al.*[9] In this study, the autolymphocytotoxic nature of the syphilitic lymphocytotoxins was formally demonstrated. Moreover, we found that these lymphocytotoxins were more frequently present in the secondary than in the primary stage of the disease.

A characteristic common to the lymphocytotoxins found in several diseases is that they are present only at a very low concentration and that there is an optimal serum dilution at which they can be detected.[19] This also applies to the syphilitic autolymphocytotoxins. For most of the reactive sera the autolymphocytotoxic reactivity could be detected or showed its maximum score at 1 μl of undiluted serum. When 3 μl of undiluted serum was used, we found a prozone effect. This result may be explained by assuming that the occupation-density of the

TABLE IV *Effect of treatment on the autolymphocytotoxic reactivity in sera from patients with early syphilis*

		Pretreatment serum		*Post-treatment serum*	
Patient No	*Stage of syphilis*	*Cytotoxic score*	*Serum dilution**	*Cytotoxic score*	*Serum dilution*
1	Primary	±	1/2	–	
2	Primary	–		–	
3	Primary	–		–	
4	Primary	+ + +	1/1	±	1/1
5	Primary	+ +	1/1	±	1/1
6	Primary	–		–	
7	Primary	+ +	1/1	±	1/1
8	Primary	+ + + +	1/2	+ + +	1/2
9	Secondary	±	1/1	–	1/1
10	Secondary	+	1/1	±	1/1
11	Secondary	+ +	1/1	+	1/1
12	Early latent	–		–	–

*Highest serum dilution showing this cytotoxic score
–, ±, +, + +, + + +, + + + + see table I

lymphocytotoxins on the lymphocyte surface membrane displays an optimum where good complement activation is achieved.

In view of the complement dependence of the syphilitic autolymphocytotoxin(s), it seems likely that it is an antibody and its presence in the 19S exclusion peak after Sephadex G-200 chromatography suggests that it is IgM.

The question whether or not the syphilitic lymphocytotoxins are specifically directed against certain subpopulation(s) of lymphocytes cannot be answered yet. However, in view of the fact that sera from 10 of our syphilitic patients killed 40% to almost 100% of the cells in the microlymphocytotoxic test, it is likely that the thymus-dependent lymphocytes are also involved as target cells.

Since the syphilitic lymphocytotoxins can only be detected at low temperatures, their biological function is not clear. However the possibility that they do react with lymphocytes at 37°C at a very low concentration (which may be too low to cause a cytotoxic reaction but at which they are able to influence the immune reactivity of the lymphocytes in vivo) cannot be excluded.

The depletion of lymphocytes in the thymus-dependent areas of lymph nodes that has been found in 11 of 18 patients with early syphilis[6] may be the result of the reactivity of lymphocytotoxins, working by a mechanism different from that operating at 15°C in the microlymphocytotoxic test. It may be that in spite of the temperature of 37°C the lymphocytotoxins react cytotoxically in the lymph nodes, helped by the influence of environmental factors. Another possibility is that a minimal non-cytotoxic coating of lymphocytes with lymphocytotoxins exists at 37°C, which may enhance phagocytosis of the lymphocytes. The last mechanism fits the results of Turner and Wright,[6] who found a replacement of lymphocytes by macrophages in the paracortical areas of lymph nodes.

Possibly, a relation exists between the presence of lymphocytotoxins in syphilitic sera and the inhibition of the PHA-response caused by plasma from these patients.[7] This inhibition was found with autologous as well as with allogeneic lymphocytes and was greater with plasma from patients with secondary syphilis than with plasma from patients with primary syphilis. This parallels our finding that the lymphocytotoxins react with autologous as well as with allogeneic cells and that they are more frequently found and show higher cytotoxic scores in sera from patients with secondary syphilis than in sera from patients with primary syphilis.

It appeared that sera with high antibody titres in the RPCFT or Kolmer reaction also had a high frequency of cold autolymphocytotoxins. This indicates that the autolymphocytotoxic reactivity is related to the humoral immune response to *T pallidum*. If the autolymphocytotoxins regulate the immune response by the suppression of the immune reactivity of the thymus-dependent lymphocytes, the relation between the autolymphocytotoxic reactivity and the humoral immune response might explain the inverse relation between the humoral and the cell-mediated immune response in primary and secondary syphilis.

Dr J Veldkamp and Dr H E Menke from the National Institute of Public Health, Bilthoven, the Netherlands, are gratefully thanked for performing the serological tests for syphilis. We are indebted to Dr R C Noble for his helpful suggestions during the preparation of the manuscript.

References

1. Menke HE. Immuunglobulinen en thymoltroebeling bij vroege syfilis. *Thesis.* Rotterdam, 1975.
2. Delhanty JJ, Catterall RD. Immunoglobulins in syphilis. *Lancet* 1969; ii: 1099-103.
3. Marshak LC, Rothman S. Skin testing with a purified suspension of *Treponema pallidum. Am J Syph* 1951; **35**: 35-41.
4. Thivolet J, Simeray A, Roland M, Challut F. Etude de l'intradermiréaction aux suspensions de tréponèmes formolées (souche Nichols pathogène) chez les syphilitiques et les sujets normaux. *Annales de l'Institut Pasteur* 1953; **85**: 23-33.
5. Kiraly K, Ràcz I. Hautproben mit gereinigter *Treponema pallidum* (TP) Vaccine. *Arch Klin Exp Derm* 1960; **209**: 583-91.
6. Turner RD, Wright DJM. Lymphadenopathy in early syphilis. *J Pathol* 1973; **110**: 305-8.
7. Levene GM, Turk JL, Wright DJM, Grimble AGS. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. *Lancet* 1969; ii: 246-7.
8. Kantor FS. Infection, anergy and cell-mediated immunity. *New Engl J Med* 1975; **292**: 629-34.
9. Naito S, Mickey MR, Hirata A, Terasaki PI. Autolymphocytotoxins following immunization by pregnancy, transplantation and disease. *Tissue Antigens* 1971; **1**: 219-28.
10. Bøyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968; **21** suppl 97: 77-89.
11. Mittal KK, Mickey MR, Singal DP, Terasaki PI. Serotyping for homotransplantation XVIII. Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation* 1968; **6**: 913-27.
12. Mottironi VD, Terasaki PI. Lymphocytotoxins in disease. I Infectious mononucleosis, rubella and measles. In: Terasaki PI, ed. *Histocompatibility Testing,* Copenhagen: Munksgaard 1970: 301-8.
13. Kreisler MJ, Naito S, Terasaki PI. Cytotoxins in disease. V Various diseases. *Transplant Proc* 1971; **3**: 112-4.
14. Mayer S, Falkenrodt A, Tongio MM. Cold lymphocytotoxins in infections and in parasitic infestations. *Tissue Antigens* 1973; **3**: 431-8.
15. Terasaki PI, Mottironi VD, Barnett EV. Cytotoxins in disease. Autocytotoxins in lupus. *New Engl J Med* 1970; **283**: 724-8.
16. Knapp W, Pateisky K. Lymphocytotoxins in myasthenia gravis. *Z Immunitaetsforsch Immunobiol* 1972; **144**: 329-34.
17. Goldberg LS, Cunningham JE, Terasaki PI. Lymphocytotoxins and pernicious anemia. *Blood* 1972; **39**: 862-5.
18. Kreisler MJ, Hirata AA, Terasaki PI. Cytotoxins in disease. III Antibodies against lymphocytes produced by vaccination. *Transplantation* 1970; **10**: 411-5.
19. Mayer S, Falkenrodt A, Tongio MM, Berrebi A. Les lymphocytotoxines froides. Etude critique de la technique. *Pathol Biol* 1973; **21**: 967-72.

Br J Vener Dis 1980;**56**:302-3.

Concentric rings simulating tinea imbricata in secondary syphilis

A case report

P A SAROJINI, A D DHARMARATNAM, K PAVITHRAN, AND C GANGADHARAN

From the Department of Dermatology and Venereology, Medical College Hospital, Trivandrum, India

SUMMARY A female patient with secondary syphilis presented with concentric rings of papules simulating tinea imbricata. To avoid the occurrence of "ping-pong" syphilis, it is stressed that both sexual partners should be treated simultaneously.

Introduction

Syphilis has been aptly described as the "great imitator." The surface lesions of secondary syphilis may simulate many other dermatological conditions.[1] Grouping of lesions or annular configurations always brings syphilis into the differential diagnosis. In annular lesions the rings may be complete or part of the rings may be missing. They may occasionally form polycyclic or gyrate patterns,[3] but concentric lesions are extremely rare; for this reason we are reporting this case.

Case report

A 20-year-old female patient attended the dermatology and venereology department on 25 April 1979 with multiple papules on the genitalia and an annular lesion on the front of the chest of two months' duration. Her husband had had some skin lesions and ulceration of the genitalia four months previously and the Venereal Disease Research Laboratory (VDRL) test had given a positive result on his serum at a 1/64 dilution; he was diagnosed as having secondary syphilis and was treated with benzathine penicillin 2·4 megaunits intramuscularly.

At that time the patient had been examined by the same doctor and she was found to be free of syphilitic infection clinically and serologically; hence no treatment was given to her. When she presented to the department on 25 April 1979 she denied any history of extramarital sexual contact. On examination she was slightly anaemic. There were multiple greyish moist papules on the genitalia (fig 1), multiple papular and scaly lesions on the trunk, limbs, and buttocks (fig 1), pigmented macules on the palms and soles, an annular lesion on the front of the chest (fig 2), and concentric rings of papules on the back (fig 3). The hard palate showed erosions. There was no lymphadenopathy, bone involvement, or alopecia. The liver and spleen were not palpable.

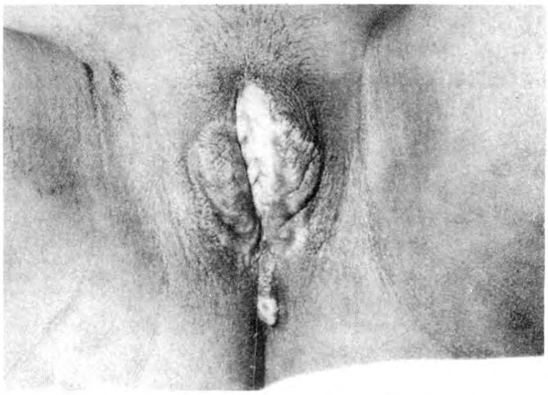


FIG 1 *Moist papules on the genitalia and papular lesions on the buttocks*

INVESTIGATIONS

Her haemoglobin was 10 g/dl. Darkground examination showed *Treponema pallidum* from the genital lesions; the serum VDRL test was reactive at a 1/8 dilution; fungus was not demonstrable by direct examination or culture of scrapings from the

Address for reprints: Dr P A Sarojini, c/o UNDP, PO Box 5580, Addis Ababa, Ethiopia

Received for publication 28 September 1979

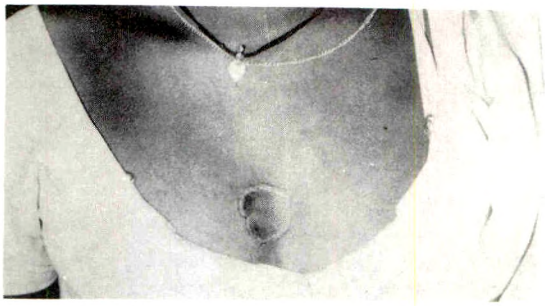


FIG 2 *Annular lesion on the front of the chest*

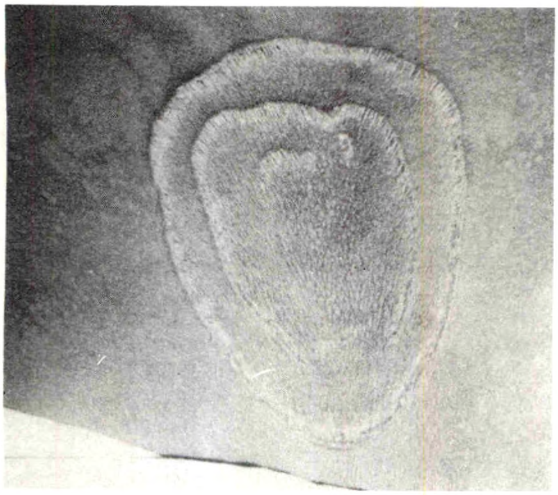


FIG 3 *Concentric rings of papules on the back*

concentric lesions. Radiographs of bones and joints of the upper and lower limbs did not show any abnormality. Chest radiographs were also normal. Biopsy of the concentric lesion showed a dense inflammatory infiltrate with lymphocytes and plasma cells in the dermis especially around the blood vessels, which showed endothelial proliferation.

The husband of this patient was examined again. He did not show any lesions and his serum VDRL test was non-reactive. Both the patient and her husband were treated with benzathine penicillin 2·4 megaunits i.m. The patient's lesions were not treated by local medication. The concentric lesions started regressing on the fifth day after treatment and disappeared completely after about two weeks. The pigmentation persisted for about two months.

Discussion

This patient presented with classical lesions of secondary syphilis, which was confirmed by the demonstration of *T pallidum* from the genital lesion and a reactive VDRL test. The concentric lesion was also due to syphilis because it resembled the annular lesion. The biopsy findings were consistent with the diagnosis of secondary syphilis and the lesion responded well to antisyphilitic treatment. Tinea imbricata was excluded from the diagnosis because of the absence of fungus and the response of the lesion to antisyphilitic treatment. Annular lesions in secondary syphilis are well known,[1-3] but concentric lesions are not mentioned in the literature. An illustration of concentric lesions in secondary syphilis is given by Rudolph and Olansky.[4] Thus, concentric rings of papules, although rare, can occur in secondary syphilis and tinea imbricata should also be included in the differential diagnosis of secondary syphilis.

In this case, because her husband had signs of secondary syphilis, the patient was also examined but not treated as she showed no evidence of infection. Later however she presented with classical secondary syphilis. The infection might have been in the incubation period at that time and the primary lesions might have been hidden or transient.

This case also illustrates the importance of treating both partners simultaneously to avoid "ping-pong" syphilis. In 1948 Schamberg and Steiger[5] reported that many apparent treatment failures were really due to reinfections.

References

1. King A, Nicol CS. *Venereal Diseases,* 2nd ed. London: Bailliere and Tindall, 1969.
2. Stokes JH, Beerman H, Ingram NR. *Modern Clinical Syphilology,* 3rd ed. Philadalphia: WB Saunders, 1944.
3. Pariser H. Infectious syphilis. *Med Clin North Am* 1964;**48**:625-36.
4. Rudolph AH, Olansky S. Syphilis. In: Demis DJ, Dobson RL, McGuire J, eds. *Clinical Dermatology.* Hagerstorm (Maryland), New York, London: Harper and Raw Publishers, 1977;6-22.
5. Shamberg IL, Steiger HP. Syphilitic relapse versus reinfection. *J Vener Dis Information* 1948;**29**:97-103.

Br J Vener Dis 1980;**56**:304-7.

Enzymatic detection of Neisseria gonorrhoeae

MILTON M TAKEGUCHI,* HOWARD H WEETALL, DAVID K SMITH,
HUGH C McDONALD, KIM A LIVSEY, CLARENCE C DETAR,
AND THOMAS A CHAPEL†

*From the *Research and Development Laboratories, Sullivan Science Park, Corning, New York, and the †Department of Syphilology and Dermatology, Wayne State University School of Medicine, Detroit, Michigan, USA*

SUMMARY In a study using a non-serological enzymatic approach for the detection of *Neisseria gonorrhoeae* in cervical and urethral swabs, the technique was shown to be technically feasible. The enzyme, 1, 2-propanediol oxidoreductase, was used as a presumptive diagnostic marker for *N gonorrhoeae*. Enzymatic activity was measured with a fluorometer. Two assay procedures were performed: (a) enzyme detection (two-tube and three-tube assays) requiring 60 minutes; and (b) enzyme inhibition (EI) (90-minute and modified 20-minute assays). Sensitivities of the two-tube, three-tube, and the 90-minute EI assays with male urethral specimens from a high-prevalence population were 80%, 84%, and 81% respectively. The specificities of these assays in a low-prevalence male population were not determined. Sensitivity of the 90-minute EI assay in a high-prevalence female group was 77% and specificity in a low-prevalence female group was 75%. The modified EI assay was tested only in a low-prevalence female group and had 87% specificity. Although the specificity of the assays needs improvement, several advantages—including early case detection, rapid availability of results, detection of current active infections, and the possibility of automation—are intrinsic in this enzymatic approach.

Introduction

Cultural isolation and identification are the most definitive diagnostic means presently available for detecting gonorrhoea in women and will very likely continue to be the principal diagnostic procedures in the foreseeable future.[1] Any test which can rapidly determine the presence of an active gonococcal infection would be an invaluable adjunct.

Recently, we reported the isolation and characterisation of a previously undescribed enzyme, 1, 2-propanediol oxidoreductase, from *Neisseria gonorrhoeae.*[2] Examination of various micro-organisms in the female genital tract showed that appreciable concentrations of this particular enzyme were present in only *Neisseria* and *Acinetobacter* species.[2] Of these organisms, *N gonorrhoeae* showed higher levels of activity than most of the other organisms. We decided therefore to use the enzymatic approach for the presumptive diagnosis of gonorrhoea in a clinical context. This report describes four variations in the use of enzymatic assays for detecting gonorrhoea in a small number of patients in three separate clinical trials.

BACTERIA

Neisseria gonorrhoeae strain F62 (obtained from Dr D S Kellogg Jr, Center for Disease Control, Atlanta, Georgia), was the strain used in lysis and sensitivity experiments.

GONOCOCCAL LYSIS

A calcium alginate-tipped swab (Inolex Corporation, Glenwood, Illinois) was used to collect gonococci growing on GC Medium (GCM, Difco Laboratories, Detroit, Michigan) supplemented with 1% IsoVitalex enrichment (Baltimore Biological Laboratories, Cockeysville, Maryland). The swab containing gonococci was then placed in 10 ml of 0·1 mol/l Tris buffer, pH 9·0, containing 0·001 mol/l cysteine for one hour at room temperature (24°C). The degree of cell lysis in this buffer was determined at various intervals by phase microscopy, scanning electron microscopy, and indirectly by standard bacteriological plate counts. Propanediol oxidoreductase activity was measured after storage for 20, 45, and 115 hours in the buffer at −20°C.

Address for reprints: Dr M M Takeguchi, Research and Development Laboratories, Sullivan Science Park, Corning Glass Works, Corning, New York 14830, USA

Received for publication 24 October 1979

DETECTION OF PROPANEDIOL OXIDOREDUCTASE

In a two-tube assay, 0·5 ml of patient sample (described below) was mixed with 2·5 ml of 0·1 mol/l Tris buffer, pH 9·0, containing 0·25 mol/l NH_4Cl, 5·02 mmol/l nicotinamide adenine dinucleotide (NAD), and 0·438 mmol/l 1, 2-propanediol. A control tube contained the same constituents with the exception of the 1, 2-propanediol. After incubation for one hour at 50°C, a fluorometer (Turner Model 111, Turner Associates, Palo Alto, California) was calibrated to zero with the control tube. The propanediol oxidoreductase activity in the test tube was then measured and expressed as fluorescence units (fu) per ml of patient sample.

A three-tube assay was also used, in which the two-tube assay was expanded to include a third tube with patient sample and Tris buffer containing NH_4Cl, NAD, and 0·362 mmol/l glycerol. The assay was conducted as above with the enzymatic activities in the propanediol and in the glycerol tubes being measured. The activity with the glycerol was then subtracted from the activity with propanediol to yield the net fu/ml with this three-tube assay.

INHIBITION OF PROPANEDIOL OXIDOREDUCTASE

Initially a 0·5-ml volume of patient sample was added to each of two tubes: one tube contained 0·1 ml normal goat serum and one tube, 0·1 ml of goat anti-propanediol oxidoreductase serum. Preparation and characterisation of this goat antiserum is described elsewhere.[3] After incubation for 30 minutes at room temperature (24°C), 2·4 ml of 0·1 mol/l Tris buffer containing 0·25 mol/l NH_4Cl, 5·02 mmol/l NAD, and 0·438 mmol/l 1, 2-propanediol were added to both tubes. These were then incubated for one hour at 50°C. A fluorometer was calibrated to zero with the immune serum tube. The activity in the normal serum tube was then measured. This assay was referred to as the 90-minute enzyme inhibition (EI) assay.

Eventually, the procedure was modified as follows to reduce the total assay time: 1·0 ml of patient sample and 0·2 ml of normal or immune goat serum were used. Both tubes were incubated for five minutes at 24°C. Then, 0·5 ml of Tris buffer containing the same concentrations of NH_4Cl, NAD, and propanediol as before were added to each tube. The tubes were incubated for 15 minutes at 50°C and read in the fluorometer as before.

PATIENT SAMPLES

Urethral swabs from 121 male patients from a population in which the prevalence of gonorrhoea was at least 32% were tested initially. These samples were collected with cotton-tipped swabs, which were first used for cultural diagnosis then for enzyme assays. Subsequent studies used separate calcium alginate swabs for culture and for enzyme assays.

Cervical swabs from 117 women in a high-prevalence (32%) group and from 100 women in a low-prevalence (2%) group were tested. All swabs were placed immediately in 2·5 ml Tris-cysteine buffer and were refrigerated until tested. Time lapse between collection of samples and testing ranged from 1·5 to five hours. Swabs were mixed in the buffer, squeezed against the inside of the tube to remove as much buffer as possible, and discarded. This buffer (or patient sample) was then subjected to the enzyme assays.

INTERPRETATION OF FLUORESCENCE UNITS

To maximise assay efficiency interpretative criteria in fluorescence units (fu) were established statistically and defined as the agreement between the enzyme assay and cultural results in a hypothetical 50/50 mixture of positive and negative samples. The criteria for each assay were: in the two-tube assay, fu $\geqslant 5$ was positive and fu $\leqslant 4$ was negative; in the three-tube assay, fu $\geqslant 2$ was positive and fu $\leqslant 1$ was negative; and in the 90-minute enzyme inhibition assay, fu $\geqslant 1$ ($\geqslant 2$ in the modified 20-minute procedure) was positive and fu $=0$ ($\leqslant 1$ in the modified procedure) was negative.

SENSITIVITY AND SPECIFICITY

The sensitivity and specificity of our assay were computed assuming the cultural method had 100% sensitivity and specificity.

CULTURE

A separate swab was used to sample simultaneously the cervix for cultural diagnosis by recommended techniques.[4]

Results

Gonococci were lysed rapidly in the Tris-cysteine buffer (table I). More than 99% of the cells were lysed in 15 minutes. Propanediol oxidoreductase activity in these lysates was detected even after prolonged storage at −20°C (table II). The effect of this Tris-cysteine buffer on the cellular integrity of other microbial species was not specifically examined in these studies. The results of testing different gonococcal concentrations with the 90-minute EI assay are shown in table III. Detectable activity ranged from 720 fu with $4{\cdot}5\times10^5$ colony forming units per test (cfu/test) to 2 fu with 450 cfu/test. Activity was not detected at concentrations equal to

TABLE I *Lysis of N gonorrhoeae in 0·1 mol/l Tris/1·0 mmol/l cysteine buffer*

Time (mins)	*Viable gonococci (cfu/ml)**	*% Lysis*
0	$4\cdot7\times10^{11}$	
5	$2\cdot1\times10^{11}$	55·32
10	$3\cdot2\times10^{10}$	93·19
15	$1\cdot8\times10^{8}$	99·96
30	$8\cdot4\times10^{6}$	>99·99
45	$7\cdot2\times10^{5}$	>99·99
60	$7\cdot8\times10^{4}$	>99·99

*Determined by standard bacteriological plate counts

TABLE II *Detection of propanediol oxidoreductase activity in gonococcal lysate*

Storage time (hours)*	*% of initial enzymatic activity*
20	40
45	40
115	27

*Storage at −20°C

TABLE III *Enzymatic activity of different gonococcal concentrations*

Gonococcal concentration (cfu)	*Enzymatic activity (fu)*
$4\cdot5\times10^{5}$	720
$4\cdot5\times10^{4}$	52
$4\cdot5\times10^{3}$	10
$4\cdot5\times10^{2}$	2
$4\cdot5\times10^{1}$	0
$4\cdot5\times10^{-1}$	0

cfu = colony forming units/test
fu = fluorescence unit determined by 90-minute enzyme inhibition assay

or less than 45 cfu/test. Similar tests with the modified enzyme inhibition assay indicated that gonococcal concentrations equal to or greater than 90 cfu could be detected.

Initial results with male urethral samples from a group with a high prevalence of gonorrhoea are shown in table IV. The three assays performed well; they correctly identified 80-84% of all culture-positive specimens and 92-97% of all culture-negative specimens.

TABLE IV *Sensitivity and specificity of three enzymatic assays for gonorrhoea in a high-prevalence male population*

Enzymatic assay	*No of samples*	*% Sensitivity*	*% Specificity*
Two-tube	121	80 (35/44)	97 (75/77)
Three-tube	121	84 (37/44)	92 (71/77)
Inhibition	89	81 (30/37)	96 (50/52)

TABLE V *Sensitivity and specificity of the 90-minute enzyme inhibition assay in a female group with a high prevalence of gonorrhoea*

Culture results	*No of samples*	*% Sensitivity*	*% Specificity*
Positive	43	77 (33/43)	
Negative	74		59 (44/74)

In subsequent trials only the EI assays and female samples were used. The 90-minute EI test results with female cervical swabs from the same high-prevalence group are summarised in table V. Thirty-three (77%) of the 43 culture-positive samples also gave positive results by the enzyme assay. In this test group, the specificity of the assay was 59%. The modified inhibition assay was not tested in this group.

However, the modified, as well as the 90-minute EI, assays were used in a female population in which the prevalence of gonorrhoea was low. The 90-minute EI assay detected 1/2 (50%) of the culture-positive samples and 74/98 (75%) of the culture-negative samples. The modified assay detected both culture-positive samples and 85/98 (87%) of the culture-negative samples.

Discussion

A definitive diagnosis of gonorrhoea is most often achieved today by bacteriological culture.[4] Screening programmes aimed at detecting asymptomatic carriers[5-7] also depend primarily on this method. Although the culture method is highly specific, it has many defects. Sensitivity is questionable, ranging anywhere from 40 to 90%. The process for obtaining specimens for culture is cumbersome and impractical for large-scale screening, and the 24 to 48 hours' incubation time required makes the method ineffective as a screening technique.[8] Furthermore, cultural screening programmes are expensive.[9]

For some time now, many investigators have been attempting to develop a serological test for gonorrhoea.[10] A good serological test may lead to more widespread screening since collection of specimens is simple, and blood samples routinely drawn for serological tests for syphilis are already available. However, many reported serological tests produce a high rate of false-positive results and also fail to detect recently infected individuals.[10] For these reasons, even serological tests are not yet ideal for screening for gonorrhoea.

In this paper, we have described a new non-serological enzymatic assay for the detection of *N gonorrhoeae*. This approach could overcome some of the major disadvantages of both the cultural method and serological tests.

Like most laboratory tests which analyse a clinical specimen, a result with this enzymatic approach depended to a large extent on the condition of the specimen examined. Initial efforts in this study were therefore concerned with the development of a reliable transport system for genital secretions. Our results indicated that the Tris-cysteine buffer was an excellent means of transporting specimens, during which time gonococci were lysed and propanediol oxidoreductase activity was maintained.

Experiments with pure gonococcal lysates suggested that this enzymatic approach was capable of detecting low concentrations of gonococci. These concentrations were within the range suggested as the required sensitivity of a test for non-viable gonococcal antigens in clinical specimens.[11]

Results of the initial trial with male specimens indicated that the sensitivity of all three assays compared favourably with many of the documented serological tests.[10] Improved sensitivity may have been observed if swabs that had not been used previously for culture had been tested.

Since a low-prevalence male group was not tested, the exact specificities of these assays were not known and accurate comparison with serological tests was not possible. However, it is reasonable to assume that male swabs will not contain the diverse types and numbers of organisms which may crossreact in the assays and which may be present in female swabs. Thus, the 92-97% specificity observed in the high-prevalence group may be indicative of the specificity in a low-prevalence male group.

In the second and third trials, we concentrated on female specimens, since this is where a rapid, non-cultural test would be most advantageous. We used the inhibition approach because we considered our goat antiserum would promote specificity and not affect sensitivity. In the high-prevalence group, the sensitivity of the 90-minute assay was 77%, which is within the range of sensitivities reported for serological tests.[10] This sensitivity is also approximately equivalent to the 80% sensitivity generally assumed for the cultural method.[8] Unfortunately, the modified assay was not tested in this population. The observed sensitivity however coupled with other advantages, such as the rapid availability of test results and the non-dependency on antibody-response time should lead to early detection and treatment of cases of gonorrhoea. Furthermore, only current active infections should be detected as persistent antigonococcal antibodies present after treatment should not interfere.

Both the 90-minute and the modified EI assays were tested in a low-prevalence female group. Although only two culture-positive samples were tested, the modified assay appeared to be more sensitive than the 90-minute assay. The specificities of the modified assay and of the 90-minute assay in this low-prevalence group were 87% and 75% respectively. Therefore, many false-positive results were observed. Possibly, many of these may not have been caused by microbial crossreactivity but were due to non-microbial factors such as cervical tissues or enzymes or by unresolved technical factors in the methodology. Alternatively, false-positive results by our assay in these comparative studies with culture results may not necessarily have all been incorrect. The sensitivity of our cultural procedure was not determined. Also, cultural results may have been falsely negative since sensitivity on culture may be as low as 40%.[8] This would have severely affected the estimate of our specificity. Nonetheless, a specificity of nearly 100% must be attained for the assay to be truly effective in screening programmes.

In summary, these studies have shown the promise of a novel and rapid approach for the detection of *N gonorrhoeae* in urethral and cervical specimens. Further studies and comprehensive clinical evaluation are needed to perfect this approach.

References

1. Gaafar HA, D'Arcangelis DC. Fluorescent antibody test for the serological diagnosis of gonorrhea. *J Clin Microbiol* 1976;**3**:438-42.
2. Takeguchi MM, Livsey KA, Detar CC, *et al.* The occurrence of a 1, 2-propanediol oxidoreductase in micro-organisms and its use as a possible diagnostic marker for *Neisseria gonorrhoeae. J Gen Microbiol* 1980 (in press).
3. McDonald HC, Takeguchi MM, Detar CC, *et al.* The isolation and characterization of a 1, 2-propanediol oxidoreductase from *Neisseria gonorrhoeae. J Gen Microbiol* 1980 (in press).
4. Kellogg DS, Holmes KK, Hill GA. Laboratory diagnosis of gonorrhea. In: Marcus S, Sherris JC eds. *Cumitech 4* Washington, DC: American Society for Microbiology. 1980.
5. Crawford G, Knapp JS, Hale J, Holmes KK. Asymptomatic gonorrhea in man: caused by gonococci with unique nutritional requirements. *Science* 1977;**196**:1352-3.
6. Pariser H. Asymptomatic gonorrhoea. *Med Clin North Am* 1972;**56**:1127-32.
7. Pariser H, Marino AF. Gonorrhea—frequently unrecognized reservoirs. *South Med J* 1970;**63**:198-201.
8. Norins LC. The case for gonococcal serology. *J Infect Dis* 1974;**130**:677-9.
9. Gaafar HA. Evaluation of the fluorescent gonococcal test—heated. *J Clin Microbiol* 1976;**4**:423-8.
10. Koransky JR, Jacobs NF. Serologic testing for gonorrhea. *Sex Transm Dis* 1977;**4**:27-31.
11. Lowe TL, Kraus SJ. Quantitation of *Neisseria gonorrhoeae* from women with gonorrhea. *J Infect Dis* 1976;**133**:621-6.

Br J Vener Dis 1980;**56**:308-10.

Sensitivity to penicillin of gonococci in different racial groups

P RODIN,* A D SETH,† D M KING,* AND A E WILKINSON†

*From the *Whitechapel Clinic and the †Venereal Diseases Reference Laboratory (PHLS), the London Hospital, London*

SUMMARY The distribution of the sensitivity to penicillin of gonococci isolated from 631 men and 290 women was analysed in relation to the racial origin of the patients and the type of source contact alleged by the men. No difference in the sensitivity patterns was found between strains from white patients from the United Kingdom and those from immigrants from the Caribbean area. Asian men harboured significantly more insensitive strains than men of other racial groups. Men of Caribbean stock who had been born in this country had significantly more infections due to fully sensitive strains. The reasons for these variations are not known, but one contributory factor may be differences within the racial groups in the proportions of infections contracted from prostitutes.

Introduction

In a nationwide study of gonorrhoea carried out in the United States between 1972 and 1975 Thornsberry *et al*[1] found a significantly higher proportion of strains which were relatively insensitive to penicillin—with minimum inhibitory concentrations (MICs) of penicillin between 0·125 and 0·25 μg/ml—among black patients than in white men and women, although the proportion of more resistant isolates (MIC $\geqslant$0·5 μg/ml) was similar in both races and sexes. Reasons suggested to account for this difference were possible selective factors, such as the tendency for relatively resistant strains to produce symptomatic infections and the degree of motivation in different racial groups to seek treatment, variations in the provision of treatment facilities in different communities, and variations in the distribution of sensitivity of gonococci to antibiotics in different geographical areas. Silver and Darling,[2] working at Bolton in the UK, found that gonococcal strains isolated from a small group of coloured immigrants were significantly less sensitive to penicillin than those from non-immigrants in the same area.

In view of these reports a retrospective study was carried out to see whether there were any differences in the distribution of sensitivity of gonococci to penicillin among patients of different racial groups who had attended a venereal disease clinic in London.

Materials and methods

Strains of *Neisseria gonorrhoeae* isolated before treatment from 631 men and 290 women who attended the Whitechapel Clinic of the London Hospital between 1976 and 1978 were tested for susceptibility to penicillin by a plate dilution method.[3] The identity of all the isolates was confirmed by an oxidase test and the production of acid from glucose but not from maltose, lactose, or saccharose. The patient's race and country of birth were noted as well as, in the men, the type of sexual contact from whom the infection was thought to have been acquired. The number of patients studied represented about 20% of the total number of patients with gonorrhoea who attended the clinic during the period reviewed. All isolates from patients attending during certain consecutive months in each of the years 1976, 1977, and 1978 were tested, but there was no other selection.

The χ^2 test was used to examine differences in the distribution of the sensitivity of strains within racial and other groups.

Results

STRAIN SENSITIVITIES

These are summarised in tables I and II. Gonococcal strains with an MIC of <0·004-0·015 μg/ml were

Address for reprints: Dr P Rodin, Whitechapel Clinic, London Hospital, London E1 1BB

Received for publication 10 December 1979

TABLE I *Sensitivity of gonococci to penicillin in racial groups (1976–78)*

		MIC (μg/ml)					
		≤0·004–0·015		*0·03–0·06*		*0·125–≥0·5*	
Racial group	*No*	*No*	*%*	*No*	*%*	*No*	*%*
Men:							
UK and Eire	227	134	59·0	49	21·6	44	19·4
UK coloured	40	33	82·5	4	10·0	3	7·5
Caribbean negro	208	125	60·1	43	20·7	40	19·3
African negro	32	13	40·6	10	31·2	9	28·1
Asian*	55	13	23·6	26	47·3	16	29·1
Miscellaneous	69	38	55·1	14	20·3	17	24·6
Total	631	356	56·4	146	23·1	129	20·4
Women:							
UK and Eire	202	122	60·4	44	21·8	36	17·8
UK coloured	20	14	70·0	3	15·0	3	15·0
Caribbean negro	53	37	69·8	9	17·0	7	13·2
African negro	4	2		2		0	
Asian*	1	0		0		1	
Miscellaneous	10	6		1		3	
Total	290	181	62·4	59	20·3	50	17·2

*Indian, Bangladeshi, and Pakistani patients

TABLE II *Distribution of sensitivity to penicillin in strains in men from different sources of infection*

			Source of infection									
	Strains		*Prostitute*		*Casual*		*Friend*		*Marital*		*Homosexual*	
MIC (μg/ml)	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*
≤0·004–0·015	356	56·4	27	48·2	234	55·1	73	63·5	12	80·0	10	50·0
0·03–0·06	146	23·1	14	25·0	105	24·7	22	19·1	3	20·0	2	10·0
0·125–≥0·5	129	20·4	15	26·8	86	20·2	20	17·4	0		8	40·0
Total	631	100	56	100	425	100	115	100	15	100	20	100

classed as fully sensitive, those with an MIC of 0·03–0·06 μg/ml as of intermediate sensitivity, and those with an MIC of ≥0·125 μg/ml as being relatively insensitive. There were no significant differences between the distribution of these three groups of strains between male or female white patients from the United Kingdom and Eire and those from the Caribbean. However, the male UK coloured patients (those of Caribbean stock who had been born and had grown up in the UK) had significantly more fully sensitive strains than white UK men ($\chi^2_1 = 8\cdot 0$, P=<0.01). No difference in the frequency of fully sensitive strains was found between the male and female UK coloured patients ($\chi^2_1 = 1\cdot 28$, P=<0·3 >0·2). Asian male patients (from India, Bangladesh, and Pakistan) were infected with significantly more intermediate and relatively insensitive strains than the other racial groups ($\chi^2_1 = 28\cdot 9$, P=<0·01). These strains were also found more frequently in the male African negroes, but when compared with the remainder of the male patients the difference was not significant at the 0·05 level.

SOURCES OF INFECTION

The sources of infection of the male patients are shown in table III. Excluding homosexually acquired infections, the Asian patients gave prostitutes as their contacts more often than the other groups ($\chi^2_1 = 28\cdot 8$, P=<0·01). Caribbean men and male UK coloured patients had relatively fewer infections from prostitutes than the male group as a whole ($\chi^2_1 = 5\cdot 5$, P=0·02; and $\chi^2_1 = 4\cdot 3$, P=<0·05 >0·02 respectively). Although there were differences in the distribution of the three sensitivity groups in the men infected by different types of female source contacts (table II), these were not significant ($\chi^2_6 = 9\cdot 3$, P=<0·3 >0·2). There was a tendency for the less sensitive strains to be found most often in the contacts of prostitutes, then in declining order, casual contacts, friends, and wives; this may perhaps reflect a gradient of promiscuity within these female groups.

Discussion

In contrast to the findings in the United States, no

TABLE III *Sources of infection in men*

		Prostitute		*Casual*†		*Friend*‡		*Marital*§		*Homosexual*	
Racial group	*No*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*	*No*	*%*
UK and Eire	227	15	6·6	139	61·2	49	21·6	8	3·5	16	7·0
UK coloured	40	0		29	72·5	10	25·0	1	2·5	0	
Caribbean negro	208	11	5·3	161	77·4	32	15·4	3	1·4	1	0·5
African negro	32	4	12·5	15	46·9	12	37·5	0		1	3·1
Asian*	55	16	29·1	34	61·8	3	5·4	2	3·6	0	
Miscellaneous	69	10	14·5	47	68·1	9	13·0	1	1·4	2	2·9
Total	631	56	8·9	425	67·3	115	18·2	15	2·4	20	3·2

*Indian, Bangladeshi, and Pakistani patients
†Relationship lasting less than one month
‡Relationship for at least one month
§Including common law wife

difference was found in the distribution of sensitivity to penicillin of gonococci recovered from UK white patients and negro immigrants from the Caribbean area. Asian men however harboured significantly more strains of reduced sensitivity. It seems likely that these findings are mainly the result of differences in social behaviour. It was at first thought that the frequency with which prostitutes were the source of infection might provide an explanation, but this was not born out when the sensitivity patterns were related to the different types of sexual contact, although there was some suggestion that it might be a contributory factor. Asian patients sometimes seek treatment from local general practitioners and it is possible that a process of antibiotic selection might have operated so that those with less sensitive strains would eventually reach the clinic when the initial treatment had failed. However, only one of the Asian men admitted having taken antibiotics shortly before his attendance. We cannot explain why UK-born coloured men had significantly more of the highly sensitive strains, but it may be relevant that none admitted to a prostitute being his source of infection.

We wish to thank the World Health Organisation for financial support.

References

1. Thornsberry C, Jaffe HW, Reynolds GH, Zaidi AA, Wiesner PJ. Patient variables associated with penicillin resistance in *Neisseria gonorrhoeae. Antimicrob Agents Chemother* 1978; **14**:327-30.
2. Silver PS, Darling WM. Penicillin insensitive gonococci in the Bolton area. Preponderance in young women and immigrants. *Br J Vener Dis* 1971; **47**:367-72.
3. Seth AD, Kolator B, Wilkinson AE. Sensitivity of *Neisseria gonorrhoeae* to antibiotics in London (1976-1978). *Br J Vener Dis* 1979; **55**:325-8.

Br J Vener Dis 1980;**56**:311-3.

Comparison of methods for the detection of penicillinase-producing Neisseria gonorrhoeae

E H SNG,* K L YEO,* V S RAJAN,† AND A L LIM*

*From the *Department of Pathology, Outram Road, and †Middle Road Hospital, Singapore*

SUMMARY In an evaluation of four methods for detecting penicillinase-producing *Neisseria gonorrhoeae* the chromogenic cephalosporin, rapid iodometric, and penicillin disc diffusion methods gave complete agreement for all the 202 strains of gonococci tested. No false-positive or false-negative results occurred. The filter paper iodometric method detected 99% of the penicillinase-producing strains without any false-positive result.

Introduction

The first report of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strains appeared in 1976. Since then a number of different tests have been described to detect such strains. These include the rapid iodometric method[1] and its filter paper modifications,[2 3] the acidometric method[4] and its microtitre modification,[5] the chromogenic cephalosporin method,[6] and various penicillin inhibition methods which use either the direct disc diffusion technique[7] or indirect techniques using penicillin-sensitive *Staphylococcus aureus*[8] or *Sarcina lutea*[9] as indicator bacteria.

A number of these tests are currently in use in different laboratories. No large-scale study has so far been reported on the relative merits of the different methods. The present study has therefore been conducted to enable laboratories to decide which of the methods may be most suitable for their use.

We have compared the chromogenic cephalosporin, rapid iodometric (tube), filter paper iodometric, and penicillin disc diffusion methods. The acidometric method has not been included because preliminary experience indicated that results were sometimes not clear cut as the penicillin-phenol red reagent tended to turn acid when stored. The indirect techniques using penicillin-sensitive indicator bacteria were also excluded because the direct disc diffusion technique is simpler.

Material and methods

Freshly isolated *N gonorrhoeae* strains were obtained from clinical specimens, which were inoculated directly on to modified Thayer-Martin medium. Identification was made by colonial morphology, oxidase reaction, and Gram-stain microscopical examination.

All isolates were first tested for the production of penicillinase by the filter paper iodometric method. Penicillinase-positive isolates and an equivalent number of penicillinase-negative strains were then tested by a different staff member using the chromogenic cephalosporin, rapid iodometric, and disc diffusion methods.

FILTER PAPER IODOMETRIC METHOD

This is a modification of the technique by Odugbemi *et al*[3]. Pieces of Whatman No 1 filter paper, each measuring approximately 5 × 6 cm, were immersed in 1% soluble starch and dried. These may be stored at room temperature for one month. When needed, the starch paper was placed in a Petri dish and soaked with a solution containing 60 000 μg/ml benzyl penicillin in phosphate-buffered saline, pH 7·3 (Oxoid). With a bacteriological loop, several colonies from a culture were spread over an area approximately 5 mm in diameter. Several different strains of gonococci may be tested on the same paper, separated from each other by approximately 1 cm. The paper was incubated at 37°C for 30 minutes with the Petri dish cover on. After incubation the paper was soaked with a solution of iodine 1 g, potassium iodine 2 g in distilled water 200 ml. The Petri dish was held at an angle of about 30° to allow the iodine solution to drain to the bottom. The starch paper turned black within seconds. The spots where the PPNG strains were smeared remained colourless.

OTHER ASSAY METHODS

The methods for the rapid iodometric, chromogenic

Address for reprints: Dr E H Sng, Department of Pathology, Outram Road, Singapore 0316, Republic of Singapore

Received for publication 8 January 1980

cephalosporin, and disc diffusion assays are as described by the WHO Scientific Group on *Neisseria gonorrhoeae* and gonococcal infections.[10] The rapid iodometric assay was performed in test tubes. The organism was incubated with penicillin solution for 60 minutes instead of 30 minutes. A positive result was indicated by the decolorisation of the starch solution within 10 minutes. The chromogenic cephalosporin assay was performed in microtitre trays, and a positive result was indicated by the development of a red colour within 30 minutes. The disc diffusion assay was performed using modified Thayer-Martin medium, and an inhibition zone size of less than 20 mm diameter was regarded as indicating a positive result.

With all the methods it was necessary to use a heavy inoculum to achieve the best results.

Results

Of a total of 202 strains tested, 99 were found to be PPNG strains. The rapid iodometric, chromogenic cephalosporin, and disc diffusion assays gave complete agreement with all the strains (table I). The filter paper iodometric method detected 99% of the PPNG strains. No false-positive result occurred with the filter paper method. Except for two strains which gave weakly positive results, the rest of the PPNG strains gave strongly positive results. With the filter paper technique, it was important to read the results immediately after the addition of the iodine, because the paper became decolorised after about 30 seconds.

TABLE I *Detection of penicillinase-producing Neisseria gonorrhoeae by different methods*

	No of strains	
Methods	+	−
Rapid iodometric	99	103
Chromogenic cephalosporin	99	103
Disc diffusion	99	103
Paper iodometric	98	104

+ Positive − negative

Both the rapid iodometric and chromogenic methods gave unequivocal results with all the strains. In a separate study 96% of 50 PPNG strains decolorised the starch in the rapid iodometric test after only 10 minutes' incubation of the organisms with the penicillin solution. One strain required 30 minutes' and another 60 minutes' incubation time. The latter strain also gave a false-negative result with the paper iodometric method.

All the PPNG strains except one turned the cephalosporin 87/312 red within two minutes of the organisms being mixed with the antibiotic. For the strain which gave a false-negative result with the filter paper iodometric method the cephalosporin took 15 minutes to turn red.

The inhibition zone sizes of the strains, using the disc diffusion method, are shown in table II. None of the PPNG strains, except one, showed any zone of inhibition around the penicillin disc. The strain which gave a negative result with the filter paper iodometric method had a zone of inhibition 16 mm in diameter. All the non-PPNG strains had zone sizes of 22 mm or more.

TABLE II *Sizes of penicillin inhibition zones of Neisseria gonorrhoeae strains*

Strains	*Size in mm*	*No*
PPNG	0	98
	16	1
Non-PPNG	22 & 23	5
	24 & 25	18
	26 & 27	23
	28 & 29	20
	≯30	37

Discussion

The detection of penicillinase-producing *N gonorrhoeae* strains requires isolation of the gonococcus. It is desirable that the method adopted by laboratories for detecting β-lactamase-production should be reliable, simple, rapid, and cheap. However, most of the current methods are fairly labour-intensive, and the disc diffusion method requires an additional day before results are known. The chromogenic cephalosporin method, being specific, sensitive, simple, and rapid, comes closest to satisfying optimum requirements. Unfortunately the reagent is not readily available and most laboratories will have to use one or more of the other methods.

In localities where the prevalence of PPNG strains is low, it is probably unnecessary to test all gonococcal isolates for β-lactamase-production routinely. It is sufficient to test strains from patients in whom treatment with penicillin has failed or who have acquired their infections from PPNG-endemic areas. For laboratories in such areas, the disc diffusion method is practical since the materials necessary for performing the test are readily available. The present study shows that there was complete agreement between the results of the disc diffusion test and the rapid iodometric or chromogenic cephalosporin tests. Even though a high proportion of the non-PPNG strains in Singapore are relatively resistant to penicillin, there was no difficulty in distinguishing the PPNG from the non-PPNG strains using a 20-mm zone size as the criterion for separating the two groups. Only one PPNG strain had a zone of inhibition and it measured 16 mm. The smallest zone size for the non-PPNG strain was 22 mm. Strains which are suspected of being PPNG should be confirmed by either the chromogenic cephalosporin or rapid iodometric methods.

Where the prevalence of PPNG strains is about 1% or more, it is likely that the treatment failure rate with penicillin will be high. In such localities it would be prudent to screen all gonococcal isolates for β-lactamase. In laboratories with a low turnover, either the chromogenic cephalosporin or rapid iodometric method is suitable. The results of the two tests showed complete agreement and were unequivocal. The reaction in the chromogenic cephalosporin assay was very rapid. A red coloration appeared within two minutes for all the PPNG stains except one; in this case, it appeared within 15 minutes. The breakdown of penicillin in the rapid iodometric method was also fast since 96% of 50 PPNG strains (in a separate study) required only 10 minutes' incubation time. However, since one strain required 30 minutes' and another 60 minutes' incubation time, it is important to adhere to the latter incubation time.

Where there is a high turnover, laboratories might consider using the simpler filter paper iodometric method. The test however requires some experience to obtain reproducible results. The original concentration of penicillin used, 100 000 μg/ml, is too strong,[3] and more specific results are obtained with 60 000 μg/ml. The iodine solution is used at 0·5% concentration. It is also important that the starch-impregnated filter paper should not be kept for longer than a month, as deterioration of the starch may also give rise to false-positive results. The principle of the method is similar to the test described by Jorgensen *et al*[2] who in addition incorporated penicillin into the starch paper and were able to keep the test paper at −20°C for a year. The prevalence of PPNG strains in Singapore was 0·2% in 1977 but rose to 10·1% in 1978. The prevalence for the first 10 months of 1979 is 18·5%. With such a high prevalence it has been necessary to screen all gonococcal isolates for β-lactamase. The simplicity of the filter paper method makes it possible to screen an average of 30 isolates a day. All positive results are confirmed by either the chromogenic cephalosporin or rapid iodometric method.

We thank Glaxo Research Ltd, England, for the compound 87/312.

References

1. Catlin BW. Iodometric detection of *Haemophilus influenzae* β-lactamase: rapid presumptive test for ampicillin resistance. *Antimicrob Agents Chemother* 1975;7:265-70.
2. Jorgensen JH, Lee JC, Alexander GA. Rapid penicillinase paper strip test for detection of β-lactamase-producing *Haemophilus influenzae* and *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1977;**11**:1087-8.
3. Odugbemi TO, Hafiz S, McEntegart MG. Penicillinase-producing *Neisseria gonorrhoeae*: detection by starch paper technique. *Br Med J* 1977;ii:500.
4. Thornsberry C, Kirven LA. Ampicillin resistance in *Haemophilus influenzae* as determined by a rapid test for β-lactamase production. *Antimicrob Agents Chemother* 1974;**6**:653-4.
5. Weissfeld AS, Sanner GD, Childress JR, *et al*. Feasibility of screening for penicillinase-producing *Neisseria gonorrhoeae* from primary culture plates by using a rapid microacidometric test. *Antimicrob Agents Chemother* 1977;**12**:703-6.
6. O'Callaghan CH, Morris A, Kirby SM, *et al*. Novel method for detection of β-lactamase by using a chromogenic cephalosporin substrate. *Antimicrob Agents Chemother* 1972;**1**:283-8.
7. World Health Organisation. *Neisseria gonorrhoeae* producing penicillinase. *WHO Wkly Epidem Rec* 1976;**38**:293-4.
8. Hodge W, Ciak J, Tramont EC. Simple method for detection of penicillinase-producing *Neisseria gonorrhoeae*. *J Clin Microbiol* 1978;**7**:102-3.
9. Martin JE Jr, Lewis JS. Selective culture screening for penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1977;**2**:605-6.
10. World Health Organisation. Techniques for the detection of β-lactamase-producing strains of *N gonorrhoeae*. *WHO Tech Rep Ser* 1978;**616**:137-42.

Br J Vener Dis 1980;**56**:314-8.

Vaginal chemoprophylaxis in the reduction of reinfection in women with gonorrhoea

Clinical evaluation of the effectiveness of a vaginal contraceptive

CLIFFORD H COLE, THOMAS G LACHER, JAMES C BAILEY, AND DIANE L FAIRCLOUGH

From the Florida Department of Health and Rehabilitative Services, Jacksonville, Florida, USA

SUMMARY A clinical investigation was undertaken to determine if chemoprophylaxis in the form of a contraceptive pessary would reduce the rate of reinfection with gonorrhoea in 1245 female patients attending a venereal disease clinic. After the introduction of the pessary the reinfection rate over a 16-week period was 19% compared with 40% in the control clinic patients. It was also found that women could be motivated to use a pessary; 65% of women used pessaries before sexual intercourse at least some of the time. High reinfection rates in venereal disease clinic patients emphasise the need for improved methods of preventing reinfection. Vaginal chemoprophylaxis appears to be an alternative means of reducing gonorrhoea morbidity.

Introduction

The increasing prevalence of gonorrhoea is widely acknowledged and of increasing concern to the health professions. Of additional concern are factors such as the developing resistance of *Neisseria gonorrhoeae* to available antibiotics and the failure of current methods, such as contact tracing, to reduce the rising incidence of infections. As a result there has recently been an increase in published reports in the use of prophylactic methods for sexually transmitted infections.[1-8]

Of the different methods of prophylaxis, vaginal preparations have received the most attention. Systemic and mechanical prophylaxis, while theoretically highly effective, have several disadvantages. The prophylactic use of systemic antibiotics may cause sensitisation with adverse reactions and also an increase in the number of resistant organisms. Although they avoid these side effects, condoms require the co-operation of the male partner and it is evident that this co-operation may not exist.[1]

As a result research has turned to the evaluation of local methods which can be used by the female partner. Of specific interest is chemoprophylaxis using commercially available products, such as vaginal contraceptives.[2-8] In-vitro studies showed that contraceptive products indeed have the potential to kill *N gonorrhoeae*.[2] Further, Lee *et al*[6] proposed that if 25% of the high-risk population consistently used a product which was 50% effective, a dramatic decrease in the prevalence of gonorrhoea could be seen within a short period of time.

These factors, together with the realisation that current management of gonorrhoea was not reducing the incidence of infections in Florida, prompted us to evaluate the potential of a chemoprophylactic vaginal contraceptive in reducing the incidence of gonorrhoea in women. The two major questions were: (a) to what extent women currently infected with gonorrhoea could be encouraged to use a pessary before sexual intercourse; and (b) whether or not chemoprophylaxis would reduce the reinfection rate in these women.

Patients and methods

This study covered two distinct phases: phase 1 (1 April to 1 October 1976) served as the pre-trial control period, during which clinic patients in two geographically separate areas (Tampa and Orlando, Florida) were managed identically. During phase 2 (1 November 1976 to 2 September 1977) vaginal pessaries were prescribed only for women attending the clinic at Tampa.

STUDY LOCATIONS

Two criteria were used to select the two study loca-

Address for reprints: Dr C H Cole, Community Tuberculosis Control Services, P O Box 210, Jacksonville, Florida 32231, USA

Received for publication 21 May 1979

tions; the first was similarity of sociodemographic characteristics based on census data (of special interest was the lower population quartile, which appears to be the commonest among venereal disease clinic patients, table I); and the second was an adequate number of patients to provide a true sample. To estimate gonorrhoea reinfection rates, having a standard error of no more than 3% with 90% confidence, at least 188 patients at each clinic during both phases had to be re-examined.

STUDY POPULATION

All women presenting at the two venereal diseases clinics with cervical gonorrhoea confirmed by isolation of gonococci on Thayer-Martin selective medium and who were treated with aqueous procaine penicillin G (APPG) 4·8 megaunits and probenecid 1 g were eligible for inclusion in the study. Patients were excluded if they were pregnant, wished to become pregnant, or were found to have syphilis. In addition, anyone not expecting to remain in the area for 90 days was excluded. Patients who were reinfected during the study were not re-entered.

CLINIC STAFF

Clinic staff were employed and trained specifically for the project. Training took place jointly for staff at both clinics and was conducted by personnel of the Florida Department of Health and Rehabilitative Services. The staff were supervised by experienced clinic supervisors, and retraining and clinic visits were performed periodically.

Trained interviewers questioned all prospective study patients to ascertain their eligibility for inclusion in the project and to record pertinent medical and sociodemographic information. In addition, information was obtained on previous history of gonorrhoea and the number of sexual partners in the last 90 days.

FOLLOW UP

Patients were asked to return for three cultures for gonococci and further interviews at 30-day intervals beginning one month after treatment (that is, after admission to the study); they were given appointment cards with dates of future attendances. During re-examination, patients attending the Tampa clinic were asked how many sexual contacts they had had since the previous clinic visit and how often they had used the pessaries. Follow-up procedures were identical in both clinics. When patients failed to keep their follow-up appointments, they were telephoned or visited at home to ensure future attendance.

Before the study, established gonorrhoea control procedures in both cities were altered in three ways. Firstly, test-of-cure cultures were not performed; secondly, contact tracing of male patients with gonorrhoea was not carried out; and, thirdly, names of male sexual partners were not elicited from female patients; referral appointment cards were however distributed to the women for their contacts to attend the clinic.

The second phase of the study differed from the first only in that chemoprophylaxis was used for patients attending the clinic at Tampa; at the initial visit patients were given 24 vaginal pessaries,† instructed in the use and preventive aspects of the product, and strongly encouraged to insert the pessaries into the vagina between 15 and 60 minutes before sexual intercourse.

ADDITIONAL USE OF ANTIBIOTICS

In case study patients might have received additional antibiotics which could have affected gonococcal isolation rates patients who were treated with any medication other than APPG 4·8 megaunits (and probenecid) for any disease or condition other than gonorrhoea were excluded from the study. At follow up patients were questioned routinely about treatment for a venereal disease received elsewhere since the last visit; those reporting such treatment were also excluded from the study.

Similarly, respiratory disease in the general population during the study period could have affected the use of antibiotics. During phase 1 and phase 2, 13% and 24% more cases of respiratory

TABLE I *Selected socioeconomic indicators for lowest population quartile* in the Tampa and Orlando Standard Metropolitan Statistical Areas (SMSA) (1970)*

SMSA	*Median education (years)*	*Median income (US$)*	*% Black population*	*% Unskilled labour*
Tampa	8·9	4846	11·1	36·0
Orlando	8·7	4198	14·9	53·3

*The lower population quartile is comprised of the population falling into the lowest 25% of the Standard Metropolitan Statistical Area (SMSA) based on socioeconomic status (SES), which is a three-factor equally-weighted index (education, income, occupation). SMSA is a US Department of Commerce, Bureau of Census term which describes a county or group of counties meeting specified population criteria.

---

†Lorophyn contraceptive suppositories, manufactured by Eaton Laboratories, Division of Norwich Pharmacal Company. The active ingredients are: phenylmercuric acetate 0·4 mg, methylbenzethonium chloride, and methylparaben in a water-dispersible base.

diseases respectively were reported in Orlando than Tampa (reported as influenza, influenza-like diseases, and streptococcal infections). This strongly suggests that the general use of antibiotics for these infections was no greater in Tampa than in Orlando.

The possibility that penicillin-resistant strains of gonococci could have affected the results of the study would appear to have been excluded. Since 1975, a surveillance programme has operated in Florida to screen all test-of-cure cultures for penicillinase-producing organisms. Only four such strains have been recovered, each associated with exposure in South-east Asia, and none was reported from Tampa or Orlando.

STATISTICAL METHODS

Life-table analysis was used to compare differences in cumulative reinfection rates at weekly intervals. This analysis was based on the methods described by Potter.[9] In this analysis the assumption is made that the risk of infection may vary with time but is constant at any given point in time. Reinfections were expressed in terms of the number of infections per 100 women. Yates's correction was not used in computing χ^2 tests because of the large size of individual data cells.

Principal components analysis was used to identify associated variables. An efficient multiple regression search routine produced a model forecasting reinfection rates.

Results

During phases 1 and 2, 570 and 675 women respectively were studied at the two clinics (table II). Sociodemographic data showed strong similarities in the patients seen at both clinics during both phases (table III). Analysis of comparative sociodemographic data showed both locations had similar study populations. The sexual behaviour of female patients was also very similar during both phases and at both locations (table IV).

The percentage of women returning for at least one follow-up visit ranged from 64% to 70%. There were no sociodemographic differences between those women who did and did not return.

REINFECTION RATES

During phase 1, the control period, the cumulative reinfection rates were similar during the first six and 42% in Orlando ($\chi^2 = 0{\cdot}01$, $P = 0{\cdot}94$).

When chemoprophylaxis was introduced in the Tampa clinic, the reinfection rate over a 16-week period fell (fig 2). The reinfection rate of 19% in Tampa was significantly lower than the 40% rate in Orlando ($\chi^2 = 2{\cdot}4$, $P = 0{\cdot}12$). Interestingly, the reinfection rates were similar during the first six weeks ($\chi^2 = 0{\cdot}00$, $P > 0{\cdot}98$), but after that period they were significantly different ($\chi^2 = 9{\cdot}46$, $P = 0{\cdot}002$).

Comparisons were also made of the reinfection rate between phase 1 and phase 2 for each clinic.

TABLE II *Culture results by phase and location of patients examined and reinfected*

	Phase 1			*Phase 2*		
	Orlando	*Tampa*	*Total*	*Orlando*	*Tampa*	*Total*
No of patients attending	280	290	570	363	312	675
No of patients recultured						
First revisit	196	190		243	200	
Second revisit	96	88		85	86	
Third revisit	48	51		30	49	
Reinfected patients:						
Positive culture results at						
First revisit	31	36		34	23	
Second revisit	17	14		11	4	
Third revisit	3	2		4	0	
Cumulative reinfection rate (at 16 weeks)	42	34		40	19	
Standard error	7	4		7	4	

TABLE III *Sociodemographic characteristics of women attending clinics at Tampa and Orlando*

	Phase 1		*Phase 2*	
	Tampa	*Orlando*	*Tampa*	*Orlando*
Mean age	21·0	20·8	20·9	20·6
Non-white (%)	63·8	53·6	50·3	52·6
Married (%)	15·2	11·1	10·3	13·5
Mean education	11·2	11·0	11·3	11·3
From low socioeconomic group (%)	59·6	64·5	50·0	57·8

TABLE IV *Comparison of sexual behaviour of study patients*

	Mean No of different sexual partners			
	Phase 1		*Phase 2*	
Visit	*Tampa*	*Orlando*	*Tampa*	*Orlando*
Initial	2·0*	1·9	2·0‡	1·9
Follow up				
First	1·0†	0·9	1·1§	1·1
Second	1·0	1·0	1·1	1·1
Third	1·1	1·0	1·1	0·9

*Excludes two women who claimed 60 and 61 male partners respectively; if included mean number would be 2·4
†Excludes one woman who claimed 33 different male partners; if included mean number would be 1·3
‡Excludes one woman who claimed 36 different male partners; if included mean number would be 2·2
§Excludes one woman who claimed 30 different male partners; if included mean number would be 1·2

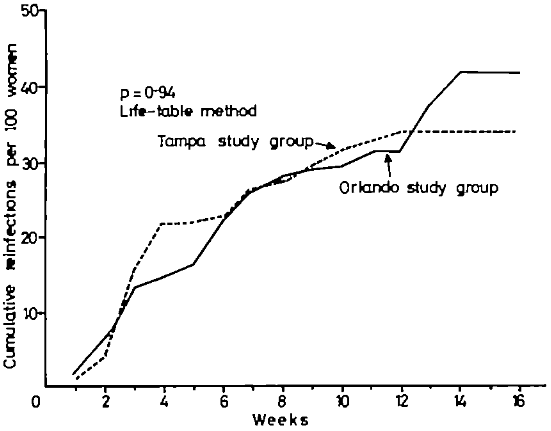


FIG 1 *Reinfections rates during phase 1 (control period) in clinic patients at Tampa compared with those at Orlando*

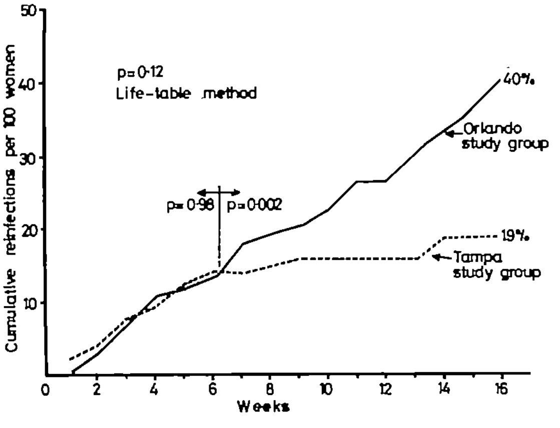


FIG 2 *Reinfection rates during phase 2 (trial period) in clinic patients at Tampa compared with those at Orlando*

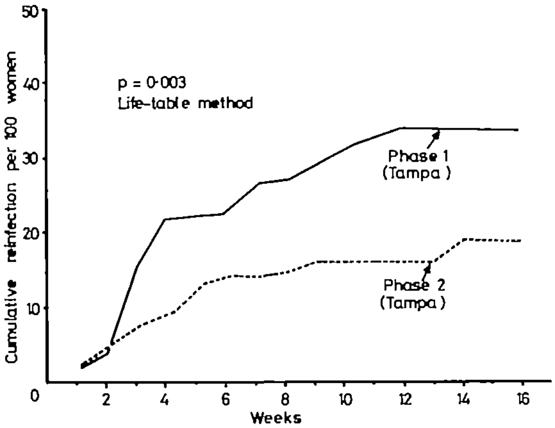


FIG 3 *Reinfection rates in clinic patients at Tampa during phase 1 (no chemoprophylaxis) compared with phase 2 (chemoprophylaxis)*

After chemoprophylaxis had been introduced in Tampa, significantly lower reinfection rates ($\chi^2 = 9{\cdot}02$, $P = 0{\cdot}003$) were recorded (fig 3). There was no difference in Orlando ($\chi^2 = 2{\cdot}17$, $P = 0{\cdot}14$) (fig 4).

STATISTICAL FINDINGS

A principal components analysis was used to show some of the characteristics of women attending the clinic in Tampa. The first factor included young women who tended not to use the pessaries and failed to return for their follow-up appointments on time. The second factor included a high rate of use of the pessary at first follow-up visit and constant use thereafter. These women were above the average age of the study group; this finding highlights the need for greater emphasis on health education in younger women.

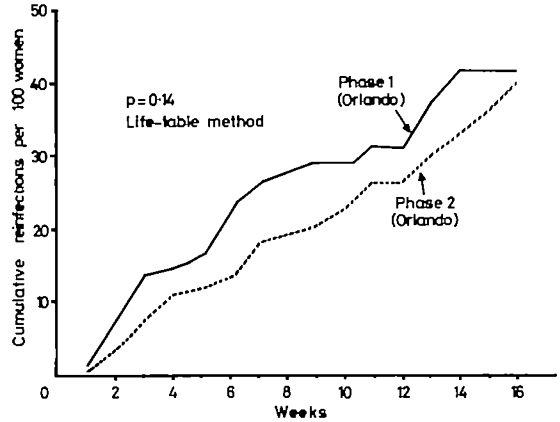


FIG 4 *Reinfection rates in clinic patients at Orlando during phase 1 (no chemoprophylaxis) compared with phase 2 (no chemoprophylaxis)*

Multiple regression analysis produced several models forecasting reinfection, the most efficient of which consisted of two variables. A greater number of sexual contacts during the project period and a lower educational level were found to be significant predictors ($P<0 \cdot 05$). In addition to showing that venereal disease education should be directed at those below the median educational level (11th grade) of study patients, this analysis indicates that clinic staff should interview patients who have a greater number of sexual contacts than average more intensively during subsequent interviews.

It was also seen that the women could be motivated to use a pessary; 65% of the women claimed that they sometimes used a pessary before sexual intercourse. The older patients used them more often than the younger ones.

The women attending the venereal disease clinic at Tampa during the study period constituted approximately 15% of the women in the greater Tampa area who were diagnosed as having gonorrhoea by all county physicians during the study. As expected the impact of reduced reinfection on reported gonorrhoea was minimal in the Tampa area during phase 2 compared with phase 1. Although previous gonorrhoea control procedures were also suspended at Orlando (control location), there was no significant change in the number of reported cases of female gonorrhoea for the greater Orlando area during the study.

Conclusion

The results of this study showed that reinfection rates were reduced when vaginal chemoprophylaxis was introduced, that women can be motivated to use a pessary, and that vaginal prophylaxis has a place in the control of gonorrhoea. These results are similar to those observed by Cutler *et al*,[8] who found that an over-the-counter vaginal contraceptive was effective in protecting against reinfection with gonorrhoea.

A third phase of the study in which chemoprophylaxis would be suspended at the Tampa clinic was planned but could not be carried out due to lack of resources. Further research in other clinics is encouraged.

The authors would like to thank John Cutler, for his assistance and encouragement; Ralph Hogan, John S Neill, John F McGarry; Michael Kerr and Edward Carson, for their patience and co-operation as clinic coordinators; William DeGrove, Mike Gardner, and Robert Taylor, for their statistical and data processing services; James Jackson, for his demographic coding skill; and Carolyn Hall, for her help in researching the topic.

References

1. Darrow WW, Wiesner PJ. Personal prophylaxis for venereal diseases. *JAMA* 1975;**233**:444-6.
2. Singh B, Cutler JC, Utidjian HMD. Studies on the development of a vaginal preparation providing both prophylaxis against venereal disease and other genital infections and contraception. II Effect in vitro of vaginal contraceptive and noncontraceptive preparations on *T pallidum* and *N gonorrhoeae*. *Br J Vener Dis* 1972;**48**:57-64.
3. Bolch CH, Warren JC. In-vitro effects of Emko on *Neisseria gonorrhoeae* and *Trichomonas vaginalis*. *Am J Obstet Gynecol* 1973;**115**:1145-8.
4. Cowan ME. A note on the susceptibility of *N gonorrhoeae* to contraceptive agent Nonyl-P. *Br J Vener Dis* 1973;**49**:65-6.
5. Donsky HJ. Nonoxynol-9 cream for genital herpes simplex. *N Engl J Med* 1979;**300**:371.
6. Lee TY, Utidjian HMD, Singh B, *et al*. Potential impact of chemical prophylaxis on the incidence of gonorrhoea. *Br J Vener Dis* 1972;**48**:376-80.
7. Cutler JC, Utidjian HMD, Singh B. Studies on the development of a vaginal preparation providing both prophylaxis against venereal disease and other genital infections and contraception. I Venereal disease prophylaxis, past experience, present status and plans for future studies. *Milit Med* 1973;**138**:88-91.
8. Cutler JC, Singh B, Carpenter U, *et al*. Vaginal contraceptives as prophylaxis against gonorrhoea and other sexually transmissible diseases. In: *Advances in Planned Parenthood*. Vol 12, No 1. Amsterdam: Excerpta Medica, 1977:45-56.
9. Potter RG. Use-effectiveness of intrauterine contraception as a problem in competing risks. In: *Family Planning in Taiwan*. New Jersey: Princeton University, 1969:458-84.

Br J Vener Dis 1980;**56:**319-21.

Diagnosis and management of anorectal gonorrhoea in women*

VALERIE A STANSFIELD
From the Middlesex Hospital Medical School, London

SUMMARY A retrospective assessment of 159 female patients who had undergone proctoscopy was carried out between January and September 1977. One hundred and twenty-seven (80%) were known contacts of gonorrhoea; of these, 63 (49·6%) were found to be infected with *Neisseria gonorrhoeae.* Of these, 29 (46%) harboured gonococci in the rectum as well as in the urethra and cervix while four (6·3%) harboured gonococci only in the rectum. Gram-stained smears gave positive results in only 12 of the 29 cases of rectal gonorrhoea, which indicates the importance of culturing rectal material. It is recommended that the management of anorectal gonorrhoea should be similar to that already established for urogenital infection.

Introduction

Anorectal gonorrhoea in women is an important and probably underdiagnosed disease. It rarely causes any symptoms or signs and is best detected by proctoscopy and microscopical examination and confirmed by culture. Studies during the last 10 years have shown anorectal infection in 40-63·7% of women diagnosed as having gonorrhoea. Parisier[1] reported that 40% of women with gonorrhoea had positive rectal culture results. Olsen[2] found positive results in rectal investigations in 63·7% of a group of 265 women who were contacts of men with gonorrhoea; in 3%, the rectal site alone gave a positive result.

Rectal investigations are not routine procedure in many clinics. Adler[3] found that they were carried out on female contacts of gonorrhoea by physicians in only 20% of clinics. It is important to know which patients are at risk from anorectal gonorrhoea and consequently where resources should be directed. In these patients with anorectal gonorrhoea, adequate follow up should be undertaken.

At James Pringle House, the Middlesex Hospital, London, proctoscopy is performed on female contacts of men treated for gonorrhoea, usually at the first visit; if omitted for any reason, it is undertaken at the next visit, particularly when urethral and cervical investigations give negative results. Patients with anorectal symptoms, or a history of anal intercourse, are also offered rectal examination. This study is a retrospective assessment by means of case records of all female patients in whom rectal investigations had been carried out during the period January to September 1977.

Patients and methods

The patients were all seen at James Pringle House, the Middlesex Hospital, London. A history was taken, including details and dates of recent sexual intercourse, contraception, and antibiotic treatment. Rectal examination was carried out with a metal proctoscope, which was introduced using a small amount of lubricating jelly. Urethral, cervical, and rectal specimens were taken with wire loops; the samples were stained by Gram's method and examined under the microscope. Specimens for culture were inoculated directly on to culture plates of a blood agar medium containing vancomycin (3 μg/ml) and colistin (8 μg/ml). The plates were put into a candle extinction jar in the clinic incubator. The full jars were transferred to the laboratory and incubated for 24 hours in candle extinction jars and then for a further 24 hours in the laboratory incubator. Plates were examined after 48 hours' incubation. Oxidase testing was carried out and oxidase-positive colonies were stained by Gram's method. Gram-negative cocci were subcultured for penicillin sensitivity and sugar fermentation tests.

Results

Proctoscopy was performed in 159 women. Of these,

---

*Paper submitted for the MSSVD Student Prize, 1979

Address for reprints: Dr V A Stansfield, 45 Brookdale Road, London E17 6QL

Received for publication 4 August 1979

127 (80%) were contacts of confirmed cases of gonorrhoea, and 63 (49·6%) had gonococci at one or more of three sites (table I). Of these, rectal infection was found in 29 (46%) and infection of the rectum alone in four (6·3%) (table II).

TABLE I *History and symptoms of female patients having contact with confirmed cases of gonorrhoea*

Symptoms/history	*No of female patients*
Anorectal	2
Vaginal and abdominal	12
Anal intercourse	5
Gonococci at one or more sites	63
Total	127

TABLE II *Sites of gonococcal infection in 63 female patients*

Site	*No*	*%*
Urethra alone	2	3·2
Cervix alone	13	20·6
Urethra + cervix	19	30·2
Total	34	54
Urethra + cervix + rectum	14	22·2
Urethra + rectum	2	3·2
Cervix + rectum	9	14·3
Rectum alone	4	6·3
Total	29	46

MICROSCOPY AND CULTURE

The results of rectal investigations and the visit at which diagnosis was made are shown in table III. Seventeen (58·6%) cultures gave positive results for *Neisseria gonorrhoeae,* although the results of the Gram-stained smears were negative. In 10 cases (34·5%), the results of both Gram-stained smears and cultures were positive, and in two (7%) the results of the smears were positive but those of the cultures were negative. In the latter two patients, one culture was overgrown with *Proteus* species and the other showed no growth, although the smear was confirmed as showing typical intracellular diplococci. Culture was essential for diagnosis; in over half the confirmed cases of gonorrhoea the results of the smears were negative. In three of the four patients with gonorrhoea affecting only the rectum, diagnosis was made by culture after proctoscopy at the second visit. In each of these patients only urethral and cervical investigations had been carried out during the first visit. In the other patient with anorectal gonorrhoea alone, positive rectal results were obtained at the first visit.

TABLE III *Results of rectal investigations*

Results		*Proctoscopy*		
Smear	*Culture*	*At 1st visit*	*At 2nd visit*	*Total*
+	−	1	1	2
+	+	8	2	10
−	+	10	7	17
Total		19	10	29

+ Positive − negative

OTHER INFECTIONS

Various other conditions were found during routine investigations (table IV). Trichomoniasis was the most common concurrent vaginal infection, being found in 17·2% of the patients with rectal gonorrhoea.

TABLE IV *Other conditions diagnosed*

	No of women	
Condition	*With gonorrhoea (all cases)*	*With anorectal gonorrhoea*
Trichomoniasis	11 (17·5%)	5 (17·2%)
Candidosis	3	1
Syphilis	1	
Herpes genitalis	1	

TREATMENT

The drugs used in the treatment of patients with gonorrhoea are given in table V. One patient was treated elsewhere, one defaulted before treatment could be given for a positive culture result, and six patients defaulted from follow up. The treatment was successful in all the remaining cases as confirmed by two sets of smears and cultures with negative results.

TABLE V *Drugs used in the treatment of gonorrhoea*

Drug	*Dosage*	*No of cases*
Benzylpenicillin	5 megaunits i m preceded by 1g oral probenecid	52
Co-trimoxazole	2 tablets twice daily for 4 days	7
Kanamycin	2g i m	2

NO HISTORY OF CONTACT WITH GONORRHOEA

The remaining 32 patients had no history of contact with gonorrhoea. Of these, 23 had anorectal symptoms or admitted to anal intercourse (table VI). The remaining nine requested examination to exclude sexually transmitted disease. Anorectal disease was diagnosed in two cases, both of these being anal infections due to *Candida albicans.*

Discussion

The overall incidence of positive results for gonorrhoea from rectal specimens in this series (46%)

TABLE VI *Symptoms of patients with no history of contact with gonorrhoea*

Symptoms	*No of patients*
Anorectal alone	11
Anorectal + anal intercourse	6
Anal intercourse alone	6
No anorectal + no anal intercourse	9
Anal candidosis	2

compares with that of Bhattacharyya and Jephcott[4] (45%) and that of Schmale and others[5] (50%).

Culture is shown to be essential in the diagnosis of rectal gonorrhoea in conjunction with microscopical examination of Gram-stained smears. Twenty-seven of the 29 specimens gave positive results by culture but only 12 by Gram-stain alone. This is probably due to the difficulty in staining and reading rectal smears.

Four patients harboured gonococci only in the rectum. If proctoscopy had not been carried out in these cases, the patients would have been considered free of infection. Only two of the 29 patients with rectal gonorrhoea had rectal symptoms and in only four proctoscopic examinations was any mucopus seen in the rectum. The patients' symptoms were anal irritation and pain on defecation.

The overall incidence of trichomoniasis (17·5%) and the similar incidence (17·2%) in patients with gonorrhoea affecting the rectum compare with an overall incidence of 19% found by Erikkson and Wanger[6] in their study on infections concurrent with gonorrhoea. How infection reaches the rectum in these patients who deny anorectal contact is still uncertain, but it seems reasonable that patients with the profuse watery vaginal discharge associated with trichomonal infection are at greater risk of urogenital gonorrhoea spreading to the rectum, as suggested by King.[7]

In this clinic, patients with urogenital gonorrhoea are discharged after treatment, if the results of two sets of smears and cultures are negative; the same procedure is followed when anorectal gonorrhoea is diagnosed. However, if rectal investigations are not carried out before treatment is given, no diagnosis of rectal gonorrhoea can be made and it is therefore impossible to make an informed decision on how many follow-up investigations are required.

This study shows that anorectal gonorrhoea is an important aspect of the disease and is present in a growing number of cases. Investigations after treatment are considered to be an essential part of the management of urogenital gonorrhoea. Anorectal gonorrhoea should be managed similarly; the disease may be excluded after two proctoscopic examinations and a cure considered when two negative results have been obtained. It is recommended that diagnostic proctoscopy be carried out in all women with gonorrhoea and in those who are contacts of patients with gonorrhoea. Management should then be the same as that for urogenital disease.

I thank the staff of James Pringle House, especially Dr R D Catterall and Professor M W Adler for their help and encouragement.

References

1. Parisier H. Asymptomatic gonorrhoea. *Med Clin North Am* 1972;**56**:1127-32.
2. Olsen GA. Value of vaginal and rectal cultures in the diagnosis of gonorrhoea. With special reference to areas with limited medical facilities. *Br J Vener Dis* 1971;**47**:102-6.
3. Adler MW. Diagnostic, treatment and reporting criteria for gonorrhoea in sexually transmitted disease clinics in England and Wales. 1 Diagnosis. *Br J Vener Dis* 1978;**54**:10-4.
4. Bhattacharyya MN, Jephcott AE. Diagnosis of gonorrhoea in women. Role of the rectal sample. *Br J Vener Dis* 1974;**50**:109-12.
5. Schmale JD, Martin JE, Domescik G. Observations on the culture diagnosis of gonorrhoea in women. *JAMA* 1969;**210**:312-7.
6. Eriksson G, Wanger L. Frequency of *N gonorrhoeae*, *T vaginalis* and *C albicans* in female venereological patients. A one-year study. *Br J Vener Dis* 1975;**51**:192-7.
7. King AJ. The complications of homosexuality. Introduction. *Proc R Soc Med* 1962;**55**:869-70.

Br J Vener Dis 1980;**56**:322-4.

Oropharyngeal flora and individual susceptibility to neisserial infection

H YOUNG,* A B HARRIS,* AND D H H ROBERTSON†

*From the *Department of Bacteriology, Edinburgh University Medical School, and the †University Department of Venereology, Royal Infirmary, Edinburgh*

SUMMARY β-haemolytic streptococci were isolated from throat swabs from 49 (10·5%) of 466 patients undergoing cultural examination for gonorrhoea. Although β-haemolytic streptococci were isolated more frequently from patients with genital or anorectal gonorrhoea (15·9%) than from those without (9·2%), the difference was not statistically significant. When groupable (A, B, C, or G) and other (non-A, -B, -C, or -G) β-haemolytic streptococci were analysed separately, a statistically significant association between non-A, -B, -C, or -G streptococci and gonococci was observed but not between groupable β-haemolytic streptococci and gonococci.

Introduction

Since the preliminary observations of Willcox *et al*,[1] it has now been convincingly demonstrated that *Neisseria meningitidis* is isolated at least twice as often from the throats of patients with genital gonorrhoea than from patients without gonorrhoea and that *Neisseria gonorrhoeae* is isolated from a genital site approximately twice as often from patients with meningococci in the throat than from patients without.[2-4] To date, only one study[5] has found no such association between genital gonorrhoea and oropharyngeal carriage of meningococci.

Ødegaard and Gedde-Dahl[4] observed that these results could indicate either that there is individual susceptibliliy to neisserial infection or that there is simply a difference in the behaviour patterns of patients who contract gonorrhoea, the conduct of the latter making them not only more likely to be infected with *N gonorrhoeae* but possibly more exposed to the acquisition of *N meningitidis* as well. We made a similar suggestion[3] and also postulated that if these results on the carriage of neisseriae merely reflect the association of intimate behaviour with the exchange of flora then oropharyngeal carriage of other "marker" organisms should show a similar correlation with genital gonorrhoea.

This paper reports our findings with β-haemolytic streptococci as marker organisms.

Address for reprints: Dr H Young, Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG

Received for publication 1 February 1980

Patients and methods

Unselected patients who attended the Department of Venereology at the Royal Infirmary, Edinburgh, for the first time during the months of September, October, November, and December 1979 were included in the survey. The sites sampled as part of the diagnostic routine, and the culture and identification methods for gonococci and meningococci, were as described previously.[3]

OROPHARYNGEAL CULTURE

An additional throat swab was taken from each patient and received at the laboratory within four hours. On receipt, each specimen was streaked on to blood agar medium (Gibco blood agar base + 5% human blood) and modified New York City (MNYC) medium.[6] Both media were incubated at 37°C in an aerobic atmosphere enriched with 10% CO_2. After incubation for 24 hours a representative colony from those plates with β-haemolytic colonies on the blood agar medium was inoculated into Todd-Hewitt broth and subcultured on to a blood agar plate. After overnight incubation the Todd-Hewitt broth culture was checked by Gram-staining and streptococci were grouped by a coagglutination method (Phadebact streptococcus test; Pharmacia Diagnostics, Uppsala, Sweden) with reagents specific for groups A, B, C, and G. The blood agar plate served as a purity check and confirmed the β-haemolytic nature of the isolate. MNYC plates were examined at 24 and 48 hours and any oxidase-positive Gram-negative diplococci were identified by standard methods.[3]

STATISTICAL METHOD

Statistical analysis of the results was made by the χ^2 method with Yates's correction.

Results

N GONORRHOEAE

Specimens from 466 patients (290 men and 176 women) were examined. *N gonorrhoeae* was isolated by culture from genital or anorectal sites of 88 (18·9%) patients (51 men and 37 women). *N gonorrhoeae* was also isolated from the throat of two of these patients and from the throat only of one additional patient.

β-HAEMOLYTIC STREPTOCOCCI

β-haemolytic streptococci (β-HS) were isolated from throat swabs of 49 (10·5%) patients (36 men and 13 women). Of the 49 β-HS isolates, two were group A, six group B, nine group C, three group G, and 29 were not A, B, C, or G.

β-HS (16 groupable and 19 non-A, -B, -C, or -G) were isolated from 35 (9·2%) of 378 patients with negative genital and ano-rectal culture results for *N gonorrhoeae*. β-HS (four groupable and 10 non-A, -B, -C, or -G) were isolated from 14 (15·9%) of 88 patients with positive genital or ano-rectal culture results for gonococci. Gonococci were isolated from 74 (17·8%) of 417 patients with negative culture results for β-HS and from 14 (28·6%) of 49 patients from whom β-HS were cultured.

ASSOCIATION OF ORGANISMS

Although β-HS were isolated more frequently from patients with than from those without gonorrhoea, and gonococci were isolated more frequently from patients with than those without oropharyngeal β-HS, the association of each organism with the other was not statistically significant (χ^2_1 2·7; 0·2> P>0·1).

When the groupable and non-A, -B, -C, or -G β-HS are analysed separately the following associations are obtained. Excluding the 29 patients with non-A, -B, -C, or -G β-HS, groupable β-HS were isolated from 16 (4·5%) of the 359 patients without gonorrhoea and from four (5·1%) of the 78 patients with gonorrhoea. Excluding the 20 patients with groupable β-HS, non-A, -B, -C, or -G β-HS were isolated from 19 (5·3%) of the 362 patients without gonorrhoea and from 10 (13·5%) of the 84 patients with gonorrhoea. The association of non-A, -B, -C, or -G β-HS with gonococci was statistically significant (χ^2_1 3·9; 0·05>P>0·02) whereas there was no statistically significant association between groupable β-HS and gonococci (χ^2_1 0·001; P>0·9).

N MENINGITIDIS

Meningococci were isolated from 17 (3·7%) of the 466 throat swabs plated in the laboratory: meningococci were isolated from seven (8%) of the 88 patients with gonorrhoea and from 10 (2·7%) of the 378 patients without gonorrhoea. The association of gonococci and meningococci was statistically significant (χ^2_1 4·3; 0·05> P>0·02).

Oropharyngeal specimens from 233 patients were examined for meningococci both by direct plating and by throat swabs sent to the laboratory: meningococci were isolated from 55 (23·6%) patients by direct plating compared with only 10 (4·3%) from swabs. The association of gonococci and meningococci in the 233 patients on whose throat specimens direct plating was done was significant (χ^2_1 6·9; P<0·01).

Discussion

Unfortunately, the question of whether or not the reported association of genital gonorrhoea and oropharyngeal carriage of meningococci[1-4] is the result of individual susceptibility to neisserial infection or is attributable to behavioural factors cannot be answered from our results. This is partly due to the complication that arises since the association of the groupable β-HS (A, B, C, and G) in patients with and without genital or anorectal gonorrhoea differs significantly from that of the non-A, -B, -C, or -G β-haemolytic streptococci. Streptococci belonging to groups A, B, C, and G are the ones most often associated with human streptococcal infection.[7] Therefore the division between groupable and non-groupable β-HS made in this study is not without justification.

Our finding of an association between non-A, -B, -C, or -G β-HS and gonorrhoea could possibly be explained as a result of quantitative differences in the oropharyngeal flora secondary to altered host resistance after infection with *N gonorrhoeae*. There are inherent difficulties in expressing what is essentially a quantitative situation by the qualitative parameters of growth or no growth on a culture plate. This is particularly true when it is borne in mind that over 90% of organisms collected on the swab will adhere so tightly that they will not be released on inoculation of the culture plate.[8]

A suitable method of quantitative bacteriological sampling will probably be required before the factors governing the association of gonococci and meningococci can be elucidated by the aid of marker oropharyngeal organisms. It is also possible that both individual susceptibility and behavioural factors contribute to the observed association making the specific contribution of each difficult to assess.

The different behaviour of the non-A, -B, -C, or -G and groupable β-HS suggests that differences might also exist between certain sero-groups and non-groupable meningococci with reference to their association with gonococci. This possibility is under investigation at present. Whether or not different sub-populations of meningococci could explain the failure of Noble *et al*[5] to demonstrate an association between gonococci and meningococci is open to speculation.

Thanks are extended to the staff of the Department of Venereology, Edinburgh Royal Infirmary, for sending clinical specimens and to Dr P W Ross and Professor J G Collee for their helpful advice during the preparation of the paper.

References

1. Willcox RR, Spencer RC, Ison C. Which *Neisseria? Br J Vener Dis* 1977;**53**:394-7.
2. Rufli Th. Which *Neisseria? Br J Vener Dis* 1978;**54**:352.
3. Young H, Harris AB, Robertson DHH. Individual susceptibility to neisserial infection? *Br J Vener Dis* 1979;**55**:188-90.
4. Ødegaard K, Gedde-Dahl TW. Frequency of simultaneous carriage of *Neisseria gonorrhoeae* and *Neisseria meningitidis. Br J Vener Dis* 1979;**55**:334-5.
5. Noble RC, Cooper RM, Miller BR. Pharyngeal colonisation by *Neisseria gonorrhoeae* and *Neisseria meningitidis* in black and white patients attending a venereal disease clinic. *Br J Vener Dis* 1979;**55**:14-19.
6. Young H. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br J Vener Dis* 1978;**54**:36-40.
7. Pollock HM, Dahlgren BJ. Distribution of streptococcal groups in clinical specimens with evaluation of bacitracin screening. *Appl Microbiol* 1974;**26**:141-3.
8. Ross PW. The isolation of *Streptococcus pyogenes* from throat swabs. *J Med Microbiol* 1977;**10**:69-76.

Br J Vener Dis 1980;**56**:325-6.

Isolation of Neisseria lactamica from the female genital tract

A case report

WILLIAM A TELFER BRUNTON,* HUGH YOUNG,* AND DONALD R K FRASER†
*From the *Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh, and †Gilmerton Road, Edinburgh*

SUMMARY *Neisseria lactamica* was isolated from the genital tract of a young patient with a persistent vaginal discharge. Although infection with *N lactamica* occurs very rarely, the importance of complete biochemical identification of neisseriae is emphasised in view of the serious social and medicolegal consequences which could result from a misdiagnosis of gonorrhoea.

Introduction

Neisseria lactamica is similar to *Neisseria meningitidis* in many respects but differs in its relative lack of virulence and ability to utilise lactose.[1] Although meningococcal colonisation of the genital tract is recognised with increasing frequency[2 3] there has only been one previous report of the isolation of *N lactamica* from a genital site.[4] This paper reports an additional isolation of *N lactamica* from the genital tract and emphasises its rarity.

Case report

CLINICAL HISTORY

An 18-year-old secretary, who was engaged to be married, presented to her general practitioner with depression and complaints of a yellow offensive vaginal discharge of some days' duration. She was taking Ovranette (Wyeth Laboratories) contraceptive pills, and her menstrual cycle had been normal. A clinical diagnosis of *Trichomonas vaginalis* infection was made and a one-week course of treatment with metronidazole (600 mg daily) started. During the week of treatment, her symptoms improved but recurred within a few days of the end of the course. Two weeks after her initial visit she returned still depressed and complaining of a troublesome vaginal discharge, dysuria, and frequency of micturition. On examination she was apyrexial but had a foul-smelling yellowish vaginal discharge. The cervix was inflamed and the uterus, although of normal size, was tender on bimanual examination. The uterine appendages were normal. Cervical and high vaginal swabs were taken, placed in Stuart's transport medium, and sent to the laboratory with a midstream specimen of urine (MSSU). Empirical treatment with clindamycin (600 mg daily) was started.

LABORATORY INVESTIGATIONS

Investigation of the MSSU showed many pus cells and *Proteus mirabilis* ($>10^8$/l). Direct microscopy of the vaginal and cervical swabs showed many pus cells and mixed organisms. No intracellular Gram-negative diplococci were seen. Routine culture yielded many *Bacteroides fragilis* and anaerobic streptococci; culture on modified New York City medium,[5] in an atmosphere of 5% CO_2 in air, showed moderate numbers of Gram-negative diplococci, which were identified as *Neisseria lactamica* by a rapid carbohydrate utilisation test.[6]

TREATMENT AND FOLLOW UP

In view of the laboratory findings, the patient was given a 10-day course of cotrimoxazole (4 tablets daily) for her urinary tract infection. When she attended for follow up two weeks later, the urinary symptoms had resolved and her MSSU culture gave a negative result. The vaginal discharge remained troublesome and two high vaginal swabs (one taken at follow up and another a month later) showed large numbers of *Bacteroides fragilis* and anaerobic streptococci. Neisseriae were not seen in or cultured from any further specimens.

Address for reprints: Dr W A Telfer Brunton, Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG

Received for publication 1 February 1980

Discussion

In view of the persistence of the vaginal discharge after the disappearance of *N lactamica* from the genital tract it is unlikely that this organism contributed to the patient's symptoms. The anaerobic streptococci and *Bacteroides fragilis* were probably more important in this respect, although their relationship to troublesome vaginal symptoms is not well understood.[7]

The rarity with which *N lactamica* is isolated from the genital tract is emphasised by our failure to isolate it from over 20 000 patients attending a sexually transmitted diseases clinic over the last five years. Nevertheless, in view of the more frequent occurrence of meningococci in the genital tract,[2 3] this report reinforces the view that full biochemical identification of oxidase-positive Gram-negative diplococci growing on selective medium is necessary to avoid the serious social and medicolegal consequences which could result from a misdiagnosis of gonorrhoea.

References

1. Gold R, Goldschneider, I, Lepow ML, Draper TF, Randolph M. Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in infants and children. *J Infect Dis* 1978; **137**: 112-21.
2. Givan KF, Thomas BW, Johnston AG. Isolation of *Neisseria meningitidis* from the urethra, cervix and anal canal: Further observations. *Br J Vener Dis* 1977; **53**: 109-12.
3. Blackwell CC, Young H, Bain RR. Isolation of *Neisseria meningitidis* and *Neisseria catarrhalis* from the genitourinary tract and anal canal. *Br J Vener Dis* 1978; **54**: 41-4.
4. Jephcott AE, Morton RS. Isolation of *Neisseria lactamica* from a genital site. *Lancet* 1972; ii: 739-40.
5. Young H. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br J Vener Dis* 1978; **54**: 36-40.
6. Young H, Paterson IC, McDonald DR. Rapid carbohydrate utilisation test for the identification of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1976; **52**: 172-5.
7. Goldacre MJ, Watt B, Loudon N, Milne LJ, Loudon JD, Vessey MP. Vaginal microbial flora in normal young women. *Br Med J* 1979; i: 1450-5.

Br J Vener Dis 1980;**56:**327-31.

Prevalence of Chlamydia trachomatis and Neisseria gonorrhoeae infections in Greenland

A seroepidemiological study

P-A MÅRDH,* I LIND,† E FROM,‡ AND A-L ANDERSEN‡

*From the *Institute of Medical Microbiology, University of Lund, Lund, Sweden; the †WHO Collaborating Centre for Reference and Research on Gonococci, Neisseria Department, Statens Seruminstitut, Copenhagen, Denmark; and the ‡Outpatient Clinic, Nuuk, Greenland*

SUMMARY In a sero-epidemiological study of the prevalence of chlamydial and gonococcal infections in Greenland three groups of subjects were studied—262 patients attending an outpatient department in the town of Nuuk (of whom 12% harboured *Chlamydia trachomatis* and 54% *Neisseria gonorrhoeae*), 63 controls from the same town, and the entire population of 150 in the settlement of Uvkusigsat. Using a microimmunofluorescence test evidence of exposure to *C trachomatis* was found in 79% of the female and 26% of the male patients, in 12% and 5% of the female and male controls respectively, and in 51% and 21% of the female and male population of Uvkusigsat respectively. Using an indirect haemagglutination test antibodies to gonococcal pili were found in sera of 92% of the female and 70% of the male patients, in 30% of the male and 10% of the female controls, and in 41% of the women and 33% of the men in Uvkusigsat. The study indicates that genital chlamydial and gonococcal infections are serious public health problems in Greenland and that such infections are acquired early in both sexes and often occur concomitantly.

Introduction

Gonorrhoea, syphilis, and (since 1977) chancroid are serious public health problems in Greenland; in 1977 the number of reported cases in a population of about 50 000 were 10 095, 674, and 975 respectively.[1] This means that morbidity for these diseases is 100-fold greater in Greenland than in Denmark. In industrialised countries, genital infections due to *Chlamydia trachomatis* probably occur at least as frequently as gonorrhoea.[2] Information about the prevalence of genital chlamydial infections in developing countries is however still scanty.

Patients attending an outpatient department in the town of Nuuk, Greenland, were examined for chlamydial and gonococcal infections by culture and serological tests. In addition, serological studies were performed both in a group of subjects from Nuuk who had no history of sexually transmitted diseases (STDs) and in the entire population of the settlement of Uvkusigsat in the district of Umanaq. On the basis of the results obtained, the value of seroepidemiological studies of STDs in developing countries is discussed.

Patients and methods

STUDY POPULATIONS

Group 1 (patients)

A total of 262 patients attending the outpatient clinic, Nuuk (Godthåb), Greenland, were studied (group 1, patients). The investigation was carried out during two periods—September to November 1977 and September to October 1978. During the first period, 97 male and 97 female patients were included in the study and during the second, 35 and 33 respectively. The age distribution of the patients, who were consecutive cases with symptoms and signs of genital infection attending the clinic during the two study periods, are given in fig 1. One-third of the men were of Danish origin compared with only one woman.

Previous gonococcal infection occurred in 92% of female and 94% of male patients. The number of earlier episodes of gonorrhoea varied from 0 to 89

Address for reprints: Dr P-A Mårdh, Institute of Medical Microbiology, Sölvegatan 23, S-223 62 Lund, Sweden

Received for publication 2 January 1980

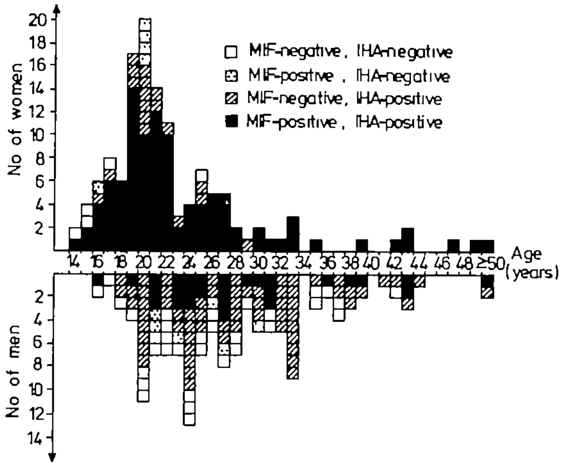


FIG 1 *Results of microimmunofluorescence (MIF) tests for IgM and IgG chlamydial antibodies and of indirect haemagglutination (IHA) tests for gonococcal pili antibodies in relation to sex and age of 262 patients (group 1)*

with a mean of eight episodes. The number of patients with current gonococcal infection is reported below. Non-gonococcal genital infections occurred in 28 (21%) of the 132 male and in 46 (35%) of the 130 female patients. Primary syphilis and chancroid were each diagnosed in eight patients whereas herpes genitalis and salpingitis of unknown aetiology each occurred in a further two patients. No evidence of current venereal or genital infection was shown in 24 (9·2%) of the 262 patients; all 24 patients were sexual contacts of patients in whom a STD had been diagnosed.

Group 2 (controls)

For comparison, 63 subjects (20 men and 43 women) from the Nuuk region, without any history of STD, were studied (group 2, controls). Eighteen of the 20 men and 16 of 27 women were of Danish origin.

Group 3 (controls)

In addition, the entire population (80 men and 70 women) of Uvkusigsat, a settlement located far north of Nuuk on the west coast of Greenland, was studied (group 3, controls). In October 1977, blood samples had been collected from this population during a seroepidemiological survey because of a case of typhoid fever. The age distribution of the population is given in fig 2.

CULTURAL TECHNIQUES

Specimens from 194 of the 262 patients (group 1, first period) attending the outpatient clinic in Nuuk were cultured for *C trachomatis* and *N gonorrhoeae* while those from the remaining 68 patients (group 1, second period) were cultured solely for gonococci. No specimens from either group 2 or group 3 were cultured.

C trachomatis

Specimens for the isolation of chlamydia were collected from the urethra of men and from the cervix of women using a calcium-alginate swab (Medical Wire and Equipment Co, UK). The specimens were transported frozen (see also Discussion) to the laboratory in Sweden in a sucrose-phosphate buffer (2-SP) containing gentamicin 10 μg (Schering) and amphotericin B 2·5 μg (Squibb) per ml. Cycloheximide-treated McCoy cells were used for the isolation of chlamydia according to a method described elsewhere,[3] except that RPMI 1640 was used as cell culture medium. The cells were cultured on coverslips 13 mm in diameter.

N gonorrhoeae

Specimens for the culture of gonococci were collected from the urethra of men and from the urethra, cervix, and rectum of women. All samples were collected using charcoal-impregnated swabs and immediately inoculated on to a selective chocolate agar medium.[4] *N gonorrhoeae* was identified by

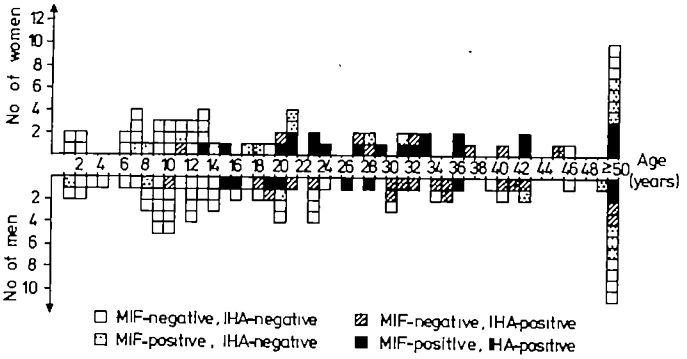


FIG 2 *Results of microimmunofluorescence (MIF) tests for IgM and IgG antibodies to C trachomatis and of the indirect haemagglutination (IHA) tests for gonococcal pili antibodies in relation to sex and age of 150 controls (group 3)*

colonial morphology, oxidase reaction, and Gram staining.

SEROLOGICAL TESTS

Sera from all three groups were tested for antibodies to *C trachomatis* and *N gonorrhoeae.*

C trachomatis

Serum IgG and IgM antibodies to *C trachomatis* were demonstrated by the microimmunofluorescence (MIF) test using pooled antigens according to the method described by Treharne *et al.*[5] The presence of IgM antibodies at a titre of ≥8 or IgG antibodies at a titre of ≥32 or both was considered a positive result.

N gonorrhoeae

Antibodies to gonococcal pili were determined by an indirect haemagglutination (IHA) test performed as previously described.[6] The antigen was the same as that used in two earlier studies.[7 8] Titres ≥40 were considered a positive result.

Results

CULTURE (GROUP 1)

Chlamydia were recovered from 12 (12%) of the 97 male and 11 (11%) of the 97 female patients examined in 1977. Of these 23 positive specimens, 20 showed less than 20 inclusions and three 25-40 inclusions per coverslip.

Gonococci were recovered from 84 (63%) of the 132 male and from 58 (45%) of the 130 female patients during 1977 and 1978. Of the 23 patients harbouring chlamydia, 10 (43%) also had gonorrhoea.

SEROLOGY

C trachomatis

Group 1. IgM antibodies to this organism, at a titre of ≥8, occurred as infrequently in the 132 (7%) male as in the 130 (6%) female patients. IgG antibodies at a titre of ≥32 were detectable more often in the women (79%) than in the men (26%) (table I).

Of the 23 chlamydia-positive patients, five had IgG antibodies at a titre of <32 whereas 3, 5, 2, 2, 3, and 1 had antibodies at a titre of 32, 64, 128, 256, 512, and 2048 respectively. One patient had a IgG titre of 64 and a IgM titre of 32 whereas the remaining patient had only IgM antibodies (titre 32).

Group 2. Of the 63 controls in this group none had IgM antibodies to *C trachomatis.* IgG antibodies to this organism at a titre of ≥32 occurred in one man and five women (table I).

Group 3. Of the 80 men and 70 women from Uvkusigsat 17 (21%) and 36 (51%) respectively had IgG antibodies to *C trachomatis.*

N gonorrhoeae

The results of the IHA tests for antibodies to gonococcal pili in the three groups are shown in table II.

Group 1. Positive results were found for 70% of the male and 92% of the female patients. The results of the IHA test in relation to previous or current gonococcal infections or both in the male and female patients are given in table III. Sixty-one (92%) of 66 female patients in whom gonococci could not be isolated but who had a previous history of gonorrhoea had a positive result. The corresponding figure for the male patients was 61%.

Group 2. Sera from two (10%) of the 20 male and 13 (30%) of the 43 female controls gave positive results to the IHA test.

Group 3. Positive IHA test results were found in 26 (33%) of the 80 men and in 29 (41%) of the 70 women.

TEST RESULTS IN RELATION TO AGE

The results of the MIF and IHA tests in relation to sex and age of groups 1 and 3 are given in figs 1 and 2 respectively.

Discussion

The prevalence of sexually transmitted diseases in Greenland is similar to that estimated for several

TABLE I. *Titres of IgG antibodies to C trachomatis in sera from patients (group 1), controls from Nuuk (group 2), and the population of Uvkusigsat (group 3)*

Group	*Sex*	*No*	*With antibody titre*										*With positive result (titre ≥32)*
			≤16	*32*	*64*	*128*	*256*	*512*	*1024*	*2048*	*4096*	*8192*	
1	M	132	98	18	11	3	0	2	0	0	0	0	34
	F	130	27	19	19	21	21	11	4	3	4	1	103
2	M	20	19	1	0	0	0	0	0	0	0	0	1
	F	43	38	1	3	1	0	0	0	0	0	0	5
3	M	80	63	5	7	3	2	0	0	0	0	0	17
	F	70	34	6	10	9	3	7	1	0	0	0	36

TABLE II *Titres of antibodies to gonococcal pili in sera from patients (group 1), controls from Nuuk (group 2), and the population of Uvkusigsat (group 3)*

Group	*Sex*	*No*	*With antibody titre*									*With positive result (titre ⩾40)*
			⩽20	*40*	*80*	*160*	*320*	*640*	*1280*	*2560*	*5120*	
1	M	132	39	20	25	21	13	11	1	1	1	93
	F	130	11	13	20	15	20	27	14	10	0	119
2	M	20	18	0	1	1	0	0	0	0	0	2
	F	43	30	7	4	1	0	1	0	0	0	13
3	M	80	54	6	9	6	5	0	0	0	0	26
	F	70	41	8	9	6	2	3	1	0	0	29

TABLE III *Results of indirect haemagglutination tests for gonococcal pili antibodies in 132 male and 130 female patients (group 1) from the outpatient clinic, Nuuk, in relation to history of gonorrhoea*

History of gonorrhoea		*No of patients*	*Test result*	
Current	*Previous*		*Positive**	*Negative*
Male patients:				
Yes	Yes	78	62	16
	No	5	2	3
No	Yes	46	28	18
	No	3	1	2
Total		132	93	39
Female patients:				
Yes	Yes	53	49	4
	No	5	4	1
No	Yes	66	61	5
	No	6	5	1
Total		130	119	11

*A titre of ⩾40 is considered a positive test result

developing countries. In contrast to most of these countries, Greenland has a medical infrastructure which allows a comparatively precise review of the problem.

A number of socioeconomic factors contribute to the high prevalence of STDs in Greenland, such as rapid urbanisation of the society, excessive alcohol consumption, and liberal sexual habits. Immigration of young single male guest workers from Denmark certainly influences the epidemiological pattern. The pattern for gonorrhoea in Greenland differs from that in many developed countries—that is, people over 30 years of age, and also young children between 7 and 12 years, not infrequently acquire gonococcal infection.

Until now beta-lactam antibiotics, with a few exceptions, have been prescribed to patients in Greenland in whom an STD had been diagnosed; this is a treatment which is not effective against chlamydial infections. Therefore, it should be possible to study the natural spread of chlamydial infections in this community. Of conditions associated with chlamydial infection, non-gonococcal urethritis is the most frequently diagnosed whereas lymphogranuloma venereum has not yet been encountered in Greenland.

Our attempts to diagnose chlamydial infections by culture were only partly sucessful. The low number of chlamydial inclusions found in the McCoy cell cultures may suggest that the specimens from the patients in Nuuk had been thawed during transportation to the laboratory in Sweden. Thus, the percentage of patients in whom a current chlamydial infection was found must be regarded as the minimum figure for the true frequency of such infections in the group of subjects studied. The low chlamydial isolation rate compared with the frequent finding of antibodies to the organism might also be due to an immune response that had eradicated the organisms or to the fact that chronic genital infections with only a few chlamydia and specific antibodies in urethral and cervical secretions were common in these patients. In experimental studies, antibodies to chlamydia were found to interfere with the formation of chlamydial inclusions in cell cultures.[10]

Culture studies have shown that double infections with *C trachomatis* and *N gonorrhoeae* commonly occur in STD clinic patients.[2] In Lund, Sweden, we have found 1/4-1/5 of such patients harbouring both these organisms. *N gonorrhoeae* was recovered from approximately half of the 23 patients in the present series with positive culture results for *C trachomatis*

The presence of serum IgM antibody to *C trachomatis* is associated with an early immune response to experimental genital infections in monkeys. After inoculation of *C trachomatis* into the uterine cavity or the Fallopian tubes of grivet monkeys, we found that IgM antibodies could be demonstrated for 5-8 weeks after the infection whereas IgG antibodies were still present after 2-3 months—that is, when the experiments were completed.[11] However, in STD clinic patients, the search for serum IgM antibodies to *C trachomatis* seems to have limited value in the diagnosis of current genital infections due to this organism. In the present study, all except two of the 23 chlamydia-positive patients had no IgM antibodies to *C trachomatis.* The low frequency of IgM antibodies to *C trachomatis* in the patients from Nuuk—that is, in 7% of male and 6% of female patients—might suggest that most of these subjects had been previously exposed to chlamydia. In reinfections or in long-standing infections, IgG antibodies are more likely to occur.

It may be argued which titre of IgG antibodies to *C trachomatis* should be considered "positive" in MIF tests. In the present study, which is a seroepidemiological survey, and in which we did not intend to establish the incidence of current chlamydial infections, we considered a titre of 32 to be a positive result.

Serum antibodies to *C trachomatis* occur more often in female than in male clinic patients.[9] This was also true for the outpatients in Nuuk. Thus such IgG antibodies occurred in 79% of the female but in only 26% of the male patients. Similar figures of seropositive men and women have been reported in STD clinic patients from the United Kingdom and United States.[2,12] The observed sex difference agrees with the result of serological studies of other agents causing STDs and seems to be due to a more efficient antigen stimulation in women than in men.

In the population of Uvkusigsat, serological evidence of exposure to chlamydia was demonstrated in 79% of the women over 12 years of age; the corresponding percentage in the men was 24. These findings suggest that chlamydial infection is a common occurrence in communities in Greenland.

The finding that six of the 10 women of Uvkusigsat, who were over 50 years of age, had high titres of IgG antibodies to *C trachomatis* might suggest that such antibodies may persist for many years or that women at this age are also exposed to the possibility of acquiring STDs.

The high prevalence of previous as well as current gonococcal infection in the patients in Nuuk was associated with the presence of antibodies to gonococcal pili in most of the sera in both the male (70%) and female (92%) patients.

In the controls, with one exception, such antibodies only occurred in women of local origin. These women had probably been previously exposed to gonococci. The correlation between age and the results of the IHA tests suggests that antibodies to gonococcal pili only occur in the age groups exposed to gonococcal infections and that the antibodies persist for years. These results agree with those obtained in previous studies.[7,8]

In a community with a high prevalence of STDs, the demonstration of serum antibodies to *C trachomatis* and *N gonorrhoeae* (at least with the tests used in the present study) is not a suitable diagnostic procedure for the establishment of current infections with these agents. However, the MIF and IHA tests used seem to be valuable for seroepidemiological surveys in areas where, for instance, attempts are made to establish the impact of sexually transmitted diseases on fertility.

This study was supported by a grant from WHO and by grant 16X-4509 from the Swedish Medical Research Council. The authors are grateful to Bodil Heilmann, Nuuk, Greenland, for her assistance in the collection of the specimens and for performing the gonococcal cultures. We also thank Dr K Gaarslev, Statens Seruminstitut, Copenhagen, Denmark, for supplying the sera from Uvkusigsat.

References

1. From E. Some aspects of venereal diseases in Greenland. *Br J Vener Dis* 1980;**56**:65-8.
2. Schachter J. Chlamydial infections. *N Engl J Med* 1978;**298**:428-35.
3. Ripa KT, Mårdh P-A. Isolation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cells. *J Clin Microbiol* 1977;**6**:328-31.
4. Reyn A. Laboratory diagnosis of gonococcal infections. *Bull WHO* 1965;**32**:449-69.
5. Treharne JD, Darougar S, Jones BR. Modification of the microimmunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977;**30**:510-7.
6. Reimann K, Lind I. An indirect hemagglutination test for demonstration of gonococcal antibodies using gonococcal pili as antigen. I Methodology and preliminary results. *Acta Pathol Microbiol Scand (C)* 1977;**85**:115-22.
7. Reimann K, Lind I, Andersen KE. An indirect hemagglutination test for demonstration of gonococcal antibodies using gonococcal pili as antigen. II Serological investigation of patients attending a dermatovenereological outpatients clinic in Copenhagen. *Acta Pathol Microbiol Scand (C)* 1980;**88**:155–62
8. Mårdh P-A, Buchanan T, Christensen P, Danielsson D, Lind I, Reimann K. Comparison of four serological tests for detection of gonococcal antibodies in patients with complicated gonococcal infections. In: *Immunobiology of Neisseria gonorrhoeae*. Washington DC: American Society for Microbiology, 1978:377-81.
9. Treharne JD, Darougar SD, Simmons PD, Thin RN. Rapid diagnosis of chlamydial infection of the cervix. *Br J Vener Dis* 1978;**54**:403-8.
10. Bryne GL, Moulder JW. Parasite-specified phagocytosis of *Chlamydia psittaci* and *Chlamydia trachomatis* by Lane Hela cells. *Infect Immun* 1978;**19**:598-606.
11. Ripa KT, Møller BR, Mårdh P-A, Freundt EA, Melsen F. Experimental acute salpingitis in grivet monkeys provoked by *Chlamydia trachomatis. Acta Pathol Microbiol Scand (B)* 1979;**87**:65-70.
12. Grayston JT, Wang S-P. New knowledge of chlamydia and the diseases they cause. *J Infect Dis* 1975;**132**:87-105.

Br J Vener Dis 1980;**56**:332-6.

Tetracycline in nongonococcal urethritis

Comparison of 2 g and 1 g daily for seven days

WILLIAM R BOWIE,* JOHN S YU,* ARCHANA FAWCETT,* AND HUGH D JONES†

*From the *Division of Infectious Diseases, Department of Medicine, University of British Columbia Faculty of Medicine; and the †Division of Venereal Diseases Control, Vancouver, British Columbia, Canada*

SUMMARY In a previous study treatment with minocycline 100 mg orally every day for seven days was as effective for nongonococcal urethritis (NGU) as 200 mg for seven days or 100 or 200 mg for 21 days. In this prospective, randomised study men with NGU received tetracycline either 500 mg or 250 mg four times daily for seven days. Of 200 men initially enrolled, *Chlamydia trachomatis* was isolated from 40% and *Ureaplasma urealyticum* from 48%. Eight of 10 homosexual men compared with 39 (21%) of 190 bisexual or heterosexual men had negative culture results for both *C trachomatis* and *U urealyticum* ($\chi^2 = 15 \cdot 5$, $P < 0 \cdot 0005$). *U urealyticum* was isolated more frequently from chlamydia-negative men and from men with 10 or fewer sex partners during their lifetime. Both regimens were equally effective in their in-vivo activity against *C trachomatis* and *U urealyticum*. Failure rates were similar with the two regimens. More obvious failure with purulent or profuse mucoid discharge and pyuria occurred more frequently with the 250-mg regimen (20% of 76 men on the 250-mg regimen compared with 7% of 67 men on the 500-mg regimen; $\chi^2 = 4 \cdot 45$, $P < 0 \cdot 05$). Failure occurred more frequently in men who were initially chlamydia-negative and in men in whom *U urealyticum* persisted after medication. Thus, the 250-mg regimen appeared to be as effective as the 500-mg regimen in the initial treatment of NGU. However, one-third of men had persistent or recurrent urethritis with these regimens, and there is a need for antimicrobial agents with greater in-vivo activity, especially against chlamydia-negative NGU.

Introduction

One standard treatment for nongonococcal urethritis (NGU) is tetracycline hydrochloride 500 mg four times daily for seven days. A previous study showed that minocycline 100 mg once daily for seven days was as effective as 100 mg twice daily for seven days (or 100 mg once or twice daily for 21 days).[1] Thus, a lower dose of tetracycline than 500 mg four times daily might be equally effective. This would be less expensive and would possibly produce fewer side effects. Consequently, in this study the results with tetracycline 250 mg four times daily for seven days (250-mg regimen) were compared with those with tetracycline hydrochloride 500 mg four times daily for seven days (500-mg regimen) in the treatment of NGU.

Address for reprints: Dr W R Bowie, Department of Medicine, Vancouver General Hospital, Vancouver, British Columbia, Canada V5Z 1M9

Received for publication 21 November 1979

Previous studies with tetracyclines have shown that chlamydia-positive NGU responds better to treatment than chlamydia-negative NGU.[1-3] Consequently, cultures were performed for *C trachomatis*, a known cause of 30-50% of cases of NGU, and for *Ureaplasma urealyticum*, a probable cause of some cases of NGU.[4] Response to treatment was correlated with the organism initially isolated to determine if a lower-dose regimen was less effective for a subgroup of cases. Secondary aims were to evaluate further the importance of *U urealyticum* as a cause of NGU and to evaluate the course in men who have pyuria without a purulent or profuse mucoid discharge at follow up.

Materials and methods

STUDY POPULATION

Men were seen at the Provincial Health Building Sexually Transmitted Diseases Clinic in Vancouver. The criteria for admission to the study were: the presence of notable pyuria (described below) and obvious urethral discharge, one or more symptoms of urethral discharge, urethral itch, or dysuria for one month or less, no treatment with antimicrobial agents within the preceeding month, and a negative result on Gram stain and subsequently a negative urethral culture result for *Neisseria gonorrhoeae* in men who had not voided in the previous two hours.

PRE-TREATMENT EVALUATION

At the initial visit men underwent a standardised interview and genital examination. Urethral exudate was examined by Gram stain and culture on Thayer-Martin medium to exclude gonorrhoea. A calcium alginate urethrogenital swab (Inolex, Glenwood, Illinois) was inserted into the urethra 1-2 cm beyond the fossa navicularis and was then placed in 0·2 mol/l sucrose phosphate transport medium and frozen at −70°C for subsequent isolation of *C trachomatis*. The first 10-15 ml of urine was then collected and cultured quantitatively for *U urealyticum.*' Approximately 7 ml of urine was centrifuged at 500×*g* for 10 minutes. The supernatant was discarded and the sediment suspended in 0·5 ml of residual urine. This was poured on to a slide to cover approximately 1 cm^2 and a cover slip overlaid. The slide was examined microscopically (×400 magnification) for trichomonads and cells. The presence of 20 or more polymorphonuclear leucocytes (PMNL) in at least two of five fields was considered to represent pyuria.

MICROBIOLOGY

Cultures for *C trachomatis* were performed using the method of Wentworth and Alexander.[5] All specimens which gave initially negative results were passed once; that is, if chlamydial inclusions were not detected in the initial culture, duplicate unstained vials were frozen at −70°C, thawed, and then used to inoculate fresh monolayers of McCoy cells. Cultures for *U urealyticum* were performed in bromothymol blue broth.[6] The urine was diluted 10^{-1}, 10^{-3}, and 10^{-5} in bromothymol blue broth to quantitate the *U urealyticum.*

THERAPY AND FOLLOW UP

Men were randomly assigned to either the 250-mg or the 500-mg (four times daily for seven days) treatment regimens. The men and the clinicians, but not the laboratory staff, knew which dose was being received. The men were asked to return for follow up at 10, 21, and 42 days after the start of therapy. They arrived for these visits when they had not voided for at least four hours. They were questioned about symptomatology, side effects of therapy, and interim sexual activity and were examined for urethral discharge. A urethrogenital swab was inserted 1-2 cm beyond the fossa navicularis for chlamydial cultures; the first-voided urine was examined for trichomonads and PMNL and cultured quantitatively for *U urealyticum.*

Response to treatment was divided into four categories: (1) pyuria, plus a purulent or profuse mucoid discharge, between 21±7 and 42±7 days after the start of treatment was called NGU; (2) pyuria with no, or minimal, non-purulent discharge which was present at 42±7 days after the start of therapy was called urine failure (both men with NGU and urine failure were called treatment failures); (3) pyuria with no, or minimal, non-purulent discharge at some time seven or more days after the start of treatment but which cleared by 42±7 days after the start of treatment was called slow response; and (4) no pyuria and no, or minimal, non-purulent discharge with follow up for 42±7 days after the start of treatment was called normal (men who were normal or had a slow response were called responders).

STATISTICS

The proportions of groups affected by selected variables were compared by the χ^2 test with Yates's correction.[7]

Results

ISOLATION

Two hundred men fulfilled the criteria for the study. There were no significant differences in the initial isolation rates for patients given the two regimens. The initial isolation rates for *C trachomatis* and *U urealyticum* are shown in table I according to sexual preference. Overall, *C trachomatis* was isolated from 40% and *U urealyticum* from 48% of patients. Eight of 10 homosexual men compared with 39 (21%) of 190 bisexual or heterosexual men had negative culture results for both *C trachomatis* and *U urealyticum* ($\chi^2_1 = 15 \cdot 5$, $p < 0 \cdot 0005$).

TABLE I *Initial isolation results for urethral cultures for C trachomatis and first-voided urine cultures for U urealyticum from men with nongonococcal urethritis, according to sexual preference*

Sexual preference	*No of isolates*				
	C+U+	*C+U−*	*C−U+*	*C−U−*	*Total*
Homosexual			2	8	10
Bisexual	1	1	1	2	5
Heterosexual	21	57	70	37	185
Total (%)	22 (11)	58 (29)	73 (37)	47 (24)	200

C+ = *C trachomatis*-positive; C− = *C trachomatis*-negative
U+ = *U urealyticum*-positive; U− = *U urealyticum*-negative

Initial isolation of *C trachomatis* was not correlated with the total number of partners. *U urealyticum* was isolated from 22 (28%) of 80 chlamydia-positive compared with 73 (61%) of 120 chlamydia-negative men ($\chi^2_1 = 21\cdot4$, P<0·0005) and from 39 (66%) of 59 men with 10 or fewer admitted sexual partners in their lifetime compared with 56 (40%) of 141 men with 11 or more partners in their lifetime ($\chi^2 = 11\cdot6$, P<0·001). Cultures for both *C trachomatis* and *U urealyticum* gave negative results in five (8%) of 59 men with 10 or fewer partners in their lifetime compared with 42 (30%) of 141 men with 11 or more ($\chi^2_1 = 10\cdot5$, P<0·005).

TREATMENT RESPONSE ACCORDING TO REGIMEN

Response to treatment according to the initial isolation results for *C trachomatis* and *U urealyticum* and also to the treatment regimen are shown in table II. Failure by 42±7 days after the start of treatment occurred in 28 (37%) of 76 men receiving 250 mg compared with 22 (33%) of 67 men receiving 500 mg ($\chi^2 = 0\cdot25$, not significant). However, significantly more men on the 250-mg regimen failed with notable pyuria plus a purulent or profuse mucoid discharge (NGU). NGU by 42±7 days after the start of treatment occurred in 15 (20%) of 76 men on the 250-mg regimen compared with five (7%) of 67 men on the 500-mg regimen ($\chi^2_1 = 4\cdot45$, P<0·05).

TREATMENT RESPONSE ACCORDING TO INITIAL CULTURE RESULTS

Response to therapy according to initial culture results is shown in table II. Failure occurred in 13 (22%) of 59 chlamydia-positive men compared with 37 (44%) of 84 chlamydia-negative men ($\chi^2 = 7\cdot4$, P<0·01). Pyuria plus a purulent or profuse mucoid discharge (NGU) was present at follow up by 42±7 days after the start of treatment in 0 of 59 chlamydia-positive men compared with 20 (24%) of 84 chlamydia-negative men ($\chi^2 = 14\cdot4$, P<0·0005).

NGU occurring by 21±7 days after the start of treatment was correlated with the initial isolation and subsequent persistence of *U urealyticum*. Among initially *U urealyticum*-positive men, seven (33%) of 21 men who remained *U urealyticum*-positive after treatment compared with five (9%) of 57 men who became *U urealyticum*-negative had NGU ($\chi^2 = 5\cdot4$, P<0·05). If only men without sexual re-exposure by 21±7 days after the start of treatment are examined, NGU occurred in five of eight who remained *U urealyticum*-positive compared with two of 30 who became *U urealyticum*-negative ($\chi^2_c = 14\cdot2$, P<0·0005).

Neither regimen was significantly more effective in treating NGU caused by *C trachomatis* or *U urealyticum*. Among chlamydia-positive men, failure occurred in five (17%) of 29 men on the 250-mg regimen compared with eight (27%) of 30 on the 500-mg regimen ($\chi^2 = 0\cdot76$, P>0·30). Among chlamydia-negative men, failure occurred in 23 (49%) of 47 men on the 250-mg regimen compared with 14 (38%) of 37 on the 500-mg regimen ($\chi^2 = 1\cdot03$, P>0·30). Among *U urealyticum*-positive men, failure occurred in 17 (40%) of 42 on the 250-mg regimen compared with nine (30%) of 30 on the 500-mg regimen ($\chi^2 = 0\cdot83$, P>0·30). Among *U urealyticum*-negative men, failure occurred in 11 (32%) of 34 on the 250-mg regimen compared with 13 (35%) of 37 on the 500-mg regimen ($\chi^2 = 0\cdot06$, P>0·70).

CULTURE RESULTS AT FOLLOW UP

Two men on the 500-mg regimen became chlamydia-positive at follow up (table III). One was initially chlamydia-negative and became culture-positive at the third follow-up visit. The other was initially chlamydia-positive and became culture-positive

TABLE II *Response of NGU to treatment according to initial isolation results for C trachomatis and U urealyticum and treatment regimen (250-mg or 500-mg)**

	Initial culture results				*Regimen*	
Response/category	*C+U+*	*C+U−*	*C−U+*	*C−U−*	*250-mg*	*500-mg*
Failures						
(1) NGU			12	8	15	5
(2) Urine failure	1	12	13	4	13	17
Total	1	12	25	12	28	22
Responders						
(3) Slow response	7	4	6	5	10	12
(4) Normal	10	25	23	13	38	33
Total	17	29	29	18	48	45
Total	18	41	54	30	76	67

*Both doses of tetracycline were given four times daily for seven days.
C+ = *C trachomatis*-positive; C− = *C trachomatis*-negative
U+ = *U urealyticum*-positive; U− = *U urealyticum*-negative

TABLE III *Culture results for C trachomatis and U urealyticum at follow up according to response to treatment*

	No of patients			
Response	*C+U+*	*C+U−*	*C−U+*	*C−U−*
NGU*		1	11	8
Urine failure†	1		5	24
Slow response†			7	15
Normal†			21	50

*Culture results at the time that NGU was diagnosed
†Culture results at 42±7 days after start of treatment
C+ = *C trachomatis*-positive; C− = *C trachomatis*-negative
U+ = *U urealyticum*-positive; U− = *U urealyticum*-negative

again at the second follow-up visit. Both had had sexual intercourse with new partners before the follow-up culture gave positive results. In all other men, cultures for *C trachomatis* gave negative results at follow up. Of the remaining men, 11 (58%) of 19 men with NGU, five (17%) of 29 urine failures, seven (32%) of 22 with slow response, and 21 (30%) of 71 who were normal were chlamydia-negative, *U urealyticum*-positive at follow up. The rest were chlamydia-negative, *U urealyticum*-negative. Positive culture results for *U urealyticum* were significantly more frequent in men with NGU compared with urine failure ($\chi_1^2 = 5 \cdot 1$, $P < 0 \cdot 025$), and in men with NGU compared with men with normal findings ($\chi_1^2 = 4 \cdot 42$, $P < 0 \cdot 05$). The presence of *U urealyticum* at 21±7 days among initially *U urealyticum*-positive men occurred in 13 (28%) of 46 men on the 250-mg regimen compared with eight (25%) of 32 on the 500-mg regimen ($\chi_1^2 = 0 \cdot 10$, $P > 0 \cdot 70$).

PYURIA AT FOLLOW UP

At follow up, an attempt was made to delay retreatment of men with pyuria to determine if the pyuria was a transient event or whether it would persist in the absence of treatment. All six men with pyuria plus a purulent or profuse mucoid discharge had pyuria at the next visit. Three had purulent or profuse mucoid discharge. Among 88 patients showing pyuria with no, or minimal, non-purulent discharge, pyuria was present in 60 (68%) at the next follow up. In the remaining 28 (32%) patients the urine was normal at the next visit. However, six (21%) of these 28 showed pyuria again at the next visit.

Discussion

This randomised, but not double-blind, study has shown that the 250-mg regimen of tetracycline is as effective in eradicating *C trachomatis* and *U urealyticum* as the 500-mg regimen, and that by six weeks after the start of treatment both regimens were associated with persistence or recurrence of NGU in one-third of the men studied.

It was previously shown that minocycline 100 mg once daily for seven days eradicated *C trachomatis* and produced similar clinical results by six weeks after treatment as 100 mg twice daily for seven days, and 100 mg once or twice daily for 21 days.[1] Thus the results in this study are not unexpected. They leave unanswered the question whether or not even lower doses or, better still, less frequent doses of tetracycline will be equally effective. Side effects were not a problem with either dose of tetracycline in the men in this study.

The importance of *U urealyticum* as a urethral pathogen remains uncertain. Although there are some contradictory data, there is mounting evidence that *U urealyticum* is a urethral pathogen. In three recent studies in Seattle[1 8 9] and a study in San Francisco,[10] *U urealyticum* was isolated more often from chlamydia-negative compared with chlamydia-positive men. Persistence of *U urealyticum* after treatment with sulphonamides,[8 9] aminocyclitols, rifampicin,[11] low-dose tetracycline,[12] and minocycline[1] is associated with a higher failure rate. Intraurethral inoculation of primates[13] and humans[14] is associated with urethritis. Thus, the higher rate of isolation of *U urealyticum* from chlamydia-negative men, and the presence of pyuria and a purulent or profuse mucoid discharge in a greater proportion of initially *U urealyticum*-positive men with persistent *U urealyticum* at follow up compared with men in whom *U urealyticum* was eradicated further supports the importance of *U urealyticum* as a urethral pathogen in some men from whom it is initially isolated.

The higher rate of NGU at follow up in men with persistent *U urealyticum* could be artifactual since only first-voided urine samples were cultured. In men with urethritis, intraurethreal swabs and first-voided urine samples yield similar rates of isolation of *U urealyticum* (W R Bowie, unpublished data). In some studies of men without urethritis however swabs were reported to yield *U urealyticum* more often than urine specimens.[15] It is not clear if these were specimens of first-voided urine. In our own serial studies of other men, cultures of first-voided urine specimens for *U urealyticum* have been consistent whether or not pyuria was present (W R Bowie, unpublished data).

It is concluded that the 250-mg regimen eradicated *C trachomatis,* that NGU at follow up is correlated with persistent *U urealyticum* in initially *U urealyticum*-positive men, and that the 250-mg regimen is adequate for the initial treatment of men with NGU. However, this regimen should not be used if gonorrhoea has not been excluded. As in other studies with intensive follow up, there is a disturbingly high (35%) failure rate. New medications must be evaluated in the treatment of NGU, especially chlamydia-negative NGU. Further studies are required to define the cause of chlamydia-negative and *U urealyticum*-negative NGU in heterosexual and homosexual men.

This research was supported by grants from the Medical Research Council of Canada, the George and Florence Heighway Foundation, and the British Columbia Medical Services Foundation.

The authors wish to thank E Paulse, S A Tayob, and V Carballo of the Division of Venereal Diseases Control of British Columbia for their co-operation.

References

1. Bowie WR, Floyd JF, Stimson JB, Alexander ER, Holmes KK. Double-blind comparison of two doses and two durations of minocycline therapy for nongonococcal urethritis. In: Siegenthaler W, Luthy R, eds. *Proceedings of 10th International Congress of Chemotherapy*, 18-23 September 1977, Zurich, Switzerland. Washington DC: American Society for Microbiology, 1978:186.
2. Handsfield HH, Alexander ER, Wang SP, Pedersen AHB, Holmes KK. Differences in the therapeutic response of chlamydia-positive and chlamydia-negative forms of nongonococcal urethritis. *J Amer Vener Dis Assoc* 1976;2:5-9.
3. Thambar IV, Simmons PD, Thin RN, Darougar S, Yearsley P. Double-blind comparison of two regimens in the treatment of nongonococcal urethritis. Seven-day *vs* 21-day courses of triple tetracycline (Deteclo). *Br J Vener Dis* 1979;55:284-8.
4. Bowie WR. Etiology and treatment of nongonococcal urethritis. *Sex Transm Dis* 1978;5:27-33.
5. Wentworth BB, Alexander ER. Isolation of *Chlamydia trachomatis* by use of 5-Iodo-2-deoxyuridine treated cells. *Appl Microbiol* 1974;27:912-6.
6. Robertson JA. Bromothymol blue broth. Improved medium for detection of *Ureaplasma urealyticum* (T-strain mycoplasma). *J Clin Microbiol* 1978;7:127-32.
7. Colton T. Regression and correlation. In: *Statistics in Medicine*, 1st ed. Boston, Massachusetts: Little, Brown and Co, 1974;174-7.
8. Bowie WR, Alexander ER, Floyd JF, Holmes J, Miller T, Holmes KK. Differential response of chlamydial and ureaplasma-associated urethritis to sulfafurazole (sulfisoxazole) and aminocyclitols. *Lancet* 1976;ii:1276-8.
9. Bowie WR, Wang SP, Alexander ER, *et al.* Etiology of nongonococcal urethritis; evidence for *Chlamydia trachomatis* and *Ureaplasma urealyticum*. *J Clin Invest* 1977;59:735-42.
10. Wong JL, Hines PA, Brasher MD, Rogers GT, Smith RF, Schachter J. The etiology of nongonococcal urethritis in men attending a venereal disease clinic. *Sex Transm Dis* 1977;4:4-8.
11. Coufalik ED, Taylor-Robinson D, Csonka GW. Treatment of nongonococcal urethritis with rifampicin as a means of defining the role of *Ureaplasma urealyticum*. *Br J Vener Dis* 1979;55:36-43.
12. Shepard MC. Quantitative relationship of *Ureaplasma urealyticum* to the clinical course of nongonococcal urethritis in the human male. *Les Colloques INSERM* 1974;33:375-9.
13. Bowie WR, DiGiacomo RF, Gale JL, Holmes KK. Urethral ureaplasma infection in the nonhuman primate *Macaca fascicularis*. *Br J Vener Dis* 1978;54:235-8.
14. Taylor-Robinson D, Csonka GW, Prentice MJ. Human intraurethral inoculation of ureaplasmas. *Quart J Med* 1977;46:309-26.
15. Tarr PI, Lee Y-H, Alpert S, Schumacher JR, Zinner SH, McCormack WM. Comparison of methods for the isolation of genital mycoplasmas from men. *J Infect Dis* 1976;133:419-23.

Br J Vener Dis 1980;**56**:337-40.

Subclinical pneumonia due to serotypes D-K of Chlamydia trachomatis

Case reports of two infants

ERIC M C DUNLOP,* ROGER J HARRIS,† SOHRAB DAROUGAR,‡
JOHN D TREHARNE,‡ AND SAFWAT S AL-EGAILY*

*From the *Whitechapel Clinic and the †Paediatrics Department, The London Hospital; and the ‡Virus Laboratory, WHO Collaborating Centre for Reference and Research on Trachoma and other Chlamydial Infections, Department of Clinical Ophthalmology, Institute of Ophthalmology, London*

SUMMARY Pneumonia due to serotypes D-K of *Chlamydia trachomatis* occurred in a 10-week-old baby, who had been successfully treated with chlortetracycline eye ointment for chlamydial ophthalmia neonatorum, and in a 7-week-old baby being treated for the same condition. Clinical signs of pneumonia were minimal. Such chlamydial pneumonia in infants must be underdiagnosed. Infants with chlamydial ophthalmia neonatorum are now routinely treated with erythromycin suspension by mouth in addition to chlortetracycline eye ointment.

Introduction

Pneumonia due to serotypes D-K of *Chlamydia trachomatis* in infants has been described.[1-12] The pneumonia was preceded in most cases, but not in all, by clinically obvious chlamydial ophthalmia neonatorum. The onset was gradual in the first three months of life. The features were closely spaced staccato coughs, eosinophilia, raised levels of IgM and IgG, the presence of antibodies to serotypes D-K of *C trachomatis* in serum of all and in nasopharyngeal material and tears of most, together with radiographic diffuse pneumonic consolidation and hyperinflation. Similar pneumonia has resulted from the experimental inoculation of the infant baboon.[10]

Case report (1)

INFANT

Baby J was born to an English mother and was mildly jaundiced. The birth weight was 2·75 kg. Mild conjunctivitis developed on the fourth day of life and was treated with neomycin 0·5% eye ointment four times a day after feeds. On the ninth day the conjunctivitis was purulent, so the following day the baby was seen in the Whitechapel Clinic for investigation. There was some swelling of the right upper lid and the conjunctivae of both eyes were inflamed. Conjunctival smears contained 100 polymorphonuclear leucocytes per high-power field (PMNL/hpf) (× 1000) but no organisms. No growth was obtained on culture for bacteria from both eyes, but coliforms were isolated from a vulvovaginal swab. *C trachomatis* was isolated from the pooled conjunctival specimens but cultures for chlamydia from throat, vagina, and rectum gave negative results. Local treatment with chlortetracycline 1% eye ointment four times a day after feeds was started.

MOTHER

The baby's mother (Miss N), who was aged 23 years and unmarried, was found to have cervicitis (80 PMNL/hpf), urethritis (40 PMNL/hpf) and proctitis (20 PMNL/hpf). Although cultures for chlamydia from the urethra, cervix, rectum, and throat gave negative results, antichlamydial IgG at a titre of 1/32 to serotypes D-K of *C trachomatis* was detected in her serum using a modified microimmunofluorescence (micro-IF) test.[13] No pathogen was cultured. Serological tests for syphilis, an Ayre's smear, and a lymphogranuloma venereum complement-fixation test (LGVCFT) for group antichlamydial antibody gave negative results. She was breast-feeding so was treated with erythromycin stearate 500 mg four times a day after meals for 14 days.

Inflammatory changes in mother and baby subsided promptly. Further cultures for chlamydia from the eyes and throat of Baby J and from the

Address for reprints: Dr E M C Dunlop, Whitechapel Clinic, The London Hospital, London E1 1BB

Received for publication 27 February 1980

throat, genitalia, and rectum of Miss N gave negative results on two occasions. After one week of treatment, smears from the left eye showed no inflammatory cells and those from the right eye only an occasional PMNL. After that conjunctival smears did not contain pus.

FOLLOW UP

Miss N then defaulted from observation. She was written to and returned when Baby J was 10 weeks and 4 days old. Baby J had continued to receive chlortetracycline eye ointment for more than six weeks; conjunctivitis had not returned after two weeks without ointment. In the week before attendance she had been crying excessively and had been "chesty" with "snuffling" in the evenings but with only occasional single coughs. When she was seen as an outpatient, her conjunctival smears contained epithelial cells without pus; there was no fever and no obvious clinical abnormality. Radiography however showed shadowing of the left mid-zone and right lower zone due to patchy consolidation (figure).

On the evening of admission her temperature was 37·4°C, on the second evening it was 37·5°C, and after that she was afebrile. Cultures from conjunctivae and throat for chlamydia gave negative results. Antichlamydial IgG against serotypes D-K of *C trachomatis* was detected at a titre of 1/256 using the micro-IF test. The white blood cell count was $8 \cdot 3 \times 10^9$/l ($8 \cdot 3 \times 10^3$/mm^3), neutrophilis 24%, lymphocytes 72%, and eosinophils 3%. Baby J was admitted to hospital and treated with 40 mg/kg daily of erythromycin suspension by mouth. Within four days her condition had improved; she was no longer "chesty" or "snuffling" and was feeding well. She was discharged from hospital. At the age of 13 weeks radiographs of the chest were normal.

Case report (2)

INFANT

While the report on Baby J was being prepared a second baby with chlamydial pneumonia was seen. Baby GB (birth weight 3·5 kg, 7·9 lb) was born to English parents. Her right eye became "sticky" during the first week of life, so that at 3 weeks of age she started treatment with neomycin 0·5% eye ointment but with no improvement.

At 6 weeks of age she was referred to the Whitechapel Clinic from the emergency and accident department. Both upper eyelids were slightly swollen and both conjunctivae were reddened and slightly swollen. A smear of the conjunctival material from the right eye contained 15 PMNL/hpf (× 1000) but no organisms; there were no PMNL in a smear from the left eye. *C trachomatis* was isolated from both

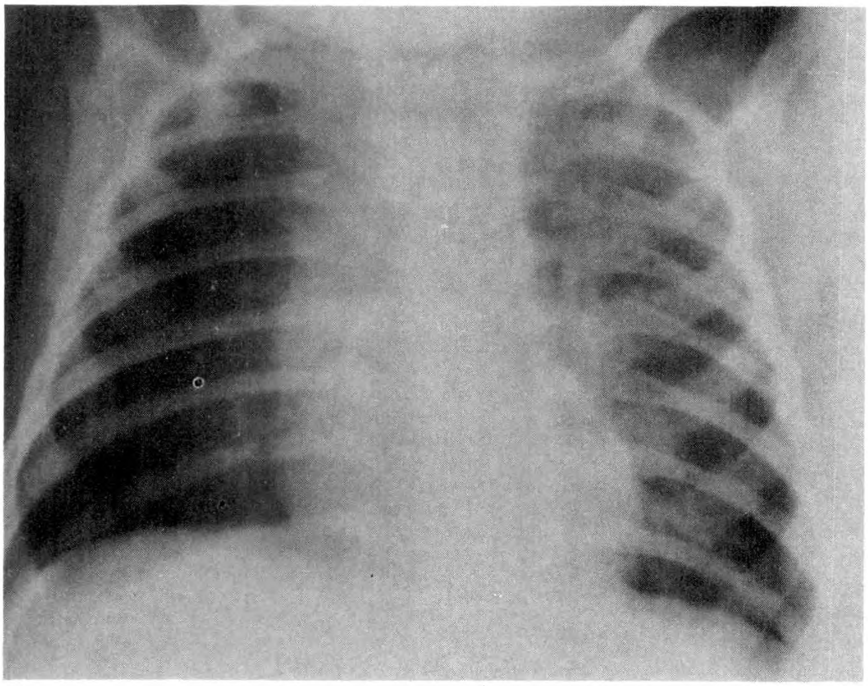


FIGURE *Radiograph of the chest of Baby J showing shadowing of left mid-zone and right lower zone* conjunctivae, the vulva, and the anorectum but not from the throat. Cultures did not show gonococci. A micro-IF test of the serum did not show antichlamydial antibodies. Treatment was changed to chlortetracycline 1% eye ointment four times a day after feeds as soon as material for these tests had been taken.

At 7 weeks the results of the cultures for chlamydia were available. Baby GB was then "chesty" at night when her breathing was audible, and she coughed occasionally. She had subsiding conjunctivitis with 20 PMNL/hpf (× 1000) in smears from both conjunctivae but no organisms. Examination of the chest showed no abnormality and she did not cough when examined. She was afebrile.

Radiography showed patchy pulmonary consolidation of the mid-zone, similar to that of Baby J. A micro-IF test did not show antichlamydial antibodies in serum or throat secretions. Treatment with erythromycin suspension 40 mg/kg daily before feeds for 14 days was started; the application of chlortetracycline 1% eye ointment was continued. A blood count showed WBC $11 \cdot 8 \times 10^9$/l ($11 \cdot 8 \times 10^3$/mm^3), eosinophilis 3%, neutrophils 11%, lymphocytes 86%, and RBC $3 \cdot 08 \times 10^{12}$/l ($3 \cdot 08 \times 10^6$/mm^3).

At 8 weeks the pneumonic consolidation was still present radiographically. At 9 weeks it had cleared and the child was clinically well. At 8 weeks the micro-IF tests showed antichlamydial IgG antibody to group 1, 2, and 3 at a titre of 1/32. No antichlamydial IgM antibody was detected.

MOTHER

Baby GB's mother was aged 24 years and married. She had had a vaginal discharge during pregnancy. Soon after delivery she had developed lower abdominal pain on the right side, followed three weeks after delivery by high abdominal pain on that side. This high abdominal pain had been associated with pain in the right shoulder, which was worse on deep breathing. She had cervicitis with an intrauterine contraceptive device in situ and a lumpy erosion. The right Fallopian tube was thickened and tender. There was tenderness around and at the edge of the liver and in the right lower abdomen. On deep breathing there was pain behind the right shoulder tip. There was no other abnormality. Her blood pressure was 110/80 mmHg.

The urethral smear contained 10 PMNL/hpf, the cervical smear 50 PMNL/hpf and red cells; the rectal smear contained debris and organisms only.

Cultures for gonococci, trichomonads, and *Candida* species and for chlamydia from the throat, cervix, urethra, and rectum gave negative results as did serological tests for syphilis and an Ayre's smear. The LGVCFT gave a positive result (1/512); antichlamydial IgG antibody to serotypes D-K was detected by the micro-IF test at a titre of 1/128. Radiography of the chest showed no abnormality.

A diagnosis of chlamydial genital infection with right salpingitis and perihepatitis was made. Because she was bottle-feeding her baby, she was treated with doxycycline 200 mg daily for 14 days. After three days the pain was less severe and after nine days it had disappeared. At the end of treatment no abnormality was detected on pelvic examination.

FATHER

The father of baby GB was seen. The last admitted extramarital sexual intercourse had been one year before attendance. He had had recurrent episodes of dysuria without obvious urethral discharge. Investigations seven hours after he had last micturated showed that he had low-grade nonspecific urethritis with 18 PMNL/hpf. Cultures for chlamydia of material from the throat and urethra (obtained with a meatal swab, an endourethral swab, and endourethral curette) gave negative results as did the micro-IF and LGVCFT. He was treated with oxytetracycline 500 mg four times a day after meals for 14 days and then defaulted from follow up.

Discussion

That Miss N had been abandoned by the father of Baby J was in keeping with the sexual instability that is usually found in the parents of babies with chlamydial and gonococcal ophthalmia neonatorum.

Mrs B had salpingitis and perihepatitis. Salpingitis commonly develops in mothers of babies with chlamydial ophthalmia neonatorum.[14-16] The isolation of *C trachomatis* from inflamed Fallopian tubes[17-19] has proved that this agent can cause salpingitis; it may cause perihepatitis (FitzHugh-Curtis syndrome).[20]

Of over 70 babies with chlamydial ophthalmia neonatorum seen by the Institute of Ophthalmology/London Hospital group, these two are the first in which chlamydial pneumonia has been diagnosed. The clinical evidence of pneumonitis was minimal and lacked the more marked illness or the characteristic cough described by Beem and Saxon.[2 3] Chlamydial pneumonia thus occurs in England, as well as in North America, and is presumably underdiagnosed.

Baby GB had chlamydia in anorectal and vulval material. As Schachter and Dawson[21] have pointed out "inclusion conjunctivitis of the newborn" is now known to be a manifestation of a more generalised chlamydial infection. Doubts about the efficacy of local treatment alone for chlamydial conjunctivitis were first expressed[22 23] after the description of the chlamydial pneumonia syndrome by Beem and Saxon.[2] These doubts were fully justified. Even if investigations show chlamydial infection of the eye alone it is clear that if treatment is confined to local therapy, this will permit chlamydial pneumonia or infection at other sites to develop. For this reason, as soon as the diagnosis of chlamydial ophthalmia neonatorum has been confirmed, erythromycin suspension 40 mg/kg daily by mouth before feeds for 14 days is now being used routinely in the treatment of affected infants in addition to chlortetracycline 1% eye ointment.

We are grateful to Dr R S Moorjani of the Radiodiagnostic Department, The London Hospital, for the radiological reports.

References

1. Schachter J, Lum L, Gooding CA, Ostler B. Pneumonitis following inclusion blenorrhoea. *J Pediatr* 1975;**87**:779-80.
2. Beem MO, Saxon EM. Respiratory tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. *N Engl J Med* 1977;**296**:306-10.
3. Beem MO, Saxon EM. Distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977:153-8.
4. Fromell GT, Bruhn FW, Schwartzman JD. Isolation of *Chlamydia trachomatis* from infant lung tissue. *N Engl J Med* 1977;**296**:1150-2.
5. Arth C, Von Schmidt B, Moses G, Schachter J. Chlamydial pneumonitis. *J Pediatr* 1978;**93**:447-9.
6. Embil JA, Ozere RL, MacDonald SW. *Chlamydia trachomatis* and pneumonia in infants: report of two cases. *Can Med Assoc J* 1978;**119**:1199-203.
7. Harrison HR, English MG, Lee CK, Alexander ER. *Chlamydia trachomatis* infant pneumonitis. Comparison with matched controls and other infant pneumonitis. *N Engl J Med* 1978;**298**:702-8.
8. Schachter J. Chlamydial infections (third of three parts). *N Engl J Med* 1978;**298**:540-9.
9. Schachter J, Dawson CR. *Human Chlamydial Infections*. Littleton, Massachusetts: PSG Publishing Company Inc, 1978.
10. Harrison HR, Alexander ER, Chiang W-T, *et al*. Experimental nasopharyngitis and pneumonia caused by *Chlamydia trachomatis* in infant baboons: Histopathological comparison with a case in a human infant. *J Infect Dis* 1979;**139**:141-6.
11. Schachter J, Grossman M, Holt J, Sweet R, Goodner E, Mills J. Prospective study of chlamydial infection in neonates. *Lancet* 1979;ii:377-80.
12. Schachter J, Grossman M, Holt J, Sweet R, Spector S. Infection with *Chlamydia trachomatis*: Involvement of multiple sites in neonates. *J Infect Dis* 1979;**139**:232-4.
13. Treharne JD, Darougar S, Jones BR. Modification of the micro-immunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977;**30**:510-7.
14. Dunlop EMC, Freedman A, Garland JA, *et al*. Infection by bedsoniae and the possibility of spurious isolation. 2 Genital infection, disease of the eye, Reiter's disease. *Am J Ophthalmol* 1967;**63**:1073-81.
15. Dunlop EMC, Goldmeier D, Darougar S, Jones BR. Chlamydial infection of the genital tract in the mothers and fathers of babies suffering from ophthalmia neonatorum due to TRIC agent. In: Catterall RD, Nichol CS, eds. *Sexually Transmitted Diseases*. London: Academic Press, 1976:83-8.
16. Rees E, Tait IA, Hobson D, Byng RE, Johnson FWA. Neonatal conjunctivitis caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. *Br J Vener Dis* 1977;**53**:173-9.
17. Hamark B, Brorsson J-E, Ellard T, Forssman L. Salpingitis and chlamydiae subgroup A. Preliminary report. *Acta Obstet Gynecol Scand* 1976;**55**:377-8.
18. Ellard T, Brorsson J-E, Hamark B, Forssman L. Isolation of chlamydia in acute salpingitis. *Scand J Infect Dis* 1976;suppl 9;82-4.
19. Mårdh P-A, Ripa KT, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977;**296**:1377-9.
20. Müller-Schoop PJW, Wang S-P, Munzinger J, Schlapfer HU, Knoblauch M, Ammann RW. *Chlamydia trachomatis* as a possible cause of peritonitis and perihepatitis in young women. *Br Med J* 1978;i:1022-4.
21. Schachter J, Dawson CR. Is trachoma an ocular component of a more generalised chlamydial infection? *Lancet* 1979;i:702-3.
22. Taylor P, Friedland P. Uncertainties in the treatment of chlamydial infections in infants. *N Engl J Med* 1977;296:1123.
23. Beem MO, Saxon EM. Uncertainties in the treatment of chlamydial infections in infants. *N Engl J Med* 1977;296:1124.

Br J Vener Dis 1980; **56:** 341-3.

Chlamydia trachomatis as a possible cause of peritonitis and perihepatitis in a young woman

J PAAVONEN* AND V V VALTONEN†

*From the *I-II Departments of Obstetrics and Gynaecology, University Central Hospital, Helsinki, and the †Department of Serology and Bacteriology, University of Helsinki, Finland*

SUMMARY In a 20-year-old woman who developed pelvic peritonitis and perihepatitis, *Chlamydia trachomatis* was isolated from the lower genital tract. There was a significant rise in the antibody titre against *C trachomatis*, suggesting that an ascending infection by this organism may have been responsible for the condition.

Introduction

The syndrome of perihepatitis is not a rare complication of gonococcal pelvic inflammatory disease (PID).[1 2] Perihepatitis associated with PID is also known as the Fitz-Hugh-Curtis syndrome.[3 4] Recently, Müller-Schoop *et al*[5] found serological evidence that *Chlamydia trachomatis* in the absence of gonococcal infection may play an important role in this peritoneal inflammation. A case of PID due to *C trachomatis* with exudative ascites and pain and tenderness in the right upper quadrant suggestive of perihepatitis in a young woman is presented.

Case report

A 20-year-old woman was admitted to hospital because of pelvic pain. The patient was para 1 with no miscarriages. She had previously been well with a regular menstrual cycle.

PAST HISTORY

She had had a normal delivery eight months previously; during pregnancy she had been examined at the outpatient clinic several times because of lower abdominal pain. Laboratory examination had shown pyuria without significant bacteriuria on three separate occasions. At 21 weeks of pregnancy she had been treated with nitrofurantoin 50 mg three times daily for three weeks. Cultures for *C trachomatis* were not performed at that time. An intrauterine device (IUD) was inserted five months after delivery.

PRESENT HISTORY

The patient's last menstrual period had occurred two weeks before admission. One week later she had had lower abdominal cramp and increased vaginal discharge. She was then admitted to hospital by her family doctor because of suspected upper genital tract infection.

On admission a gynaecologist found purulent cervicitis and adnexal tenderness on both sides. Her temperature was 36·8°C. White blood cell (WBC) count was $8 \cdot 0 \times 10^9/l$. A Papanicolaou cervicovaginal smear showed severe inflammatory changes (class II) and a large number of leucocytes. Cervical cultures for *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and yeasts all gave negative results. Cultures for *C trachomatis* were performed simultaneously using an isolation technique described elsewhere.[6] The patient was discharged and treated for suspected PID with penicillin V 2 megaunits orally four times daily and metronidazole 300 mg orally three times daily.

Four days later however the patient was readmitted because of vomiting and pelvic and upper abdominal pain. Her temperature was 37·8°C. Examination showed pelvic peritonitis and severe tenderness at the right costal margin (Murphy's sign). The WBC count was $10 \cdot 5 \times 10^9/l$, and the erythrocyte sedimentation rate (ESR) was 20 mm in the first hour. Culdocentesis produced 400 ml of yellowish serous inoffensive fluid. Culture results for *C trachomatis* from both cervical and urethral specimens were positive. Parenteral treatment with doxycycline 100 mg 12 hourly for four days was started (and continued orally for 10 days). Acute-phase titre in the single-antigen immunofluorescence test (IFAT) for IgG antichlamydial antibodies[7] was 128. Radiographs of the chest and abdomen were normal.

Address for reprints: Dr J Paavonen, I-II Departments of Obstetrics and Gynaecology, University Central Hospital, Haartmaninkatu 2, SF-00290 Helsinki 29, Finland

Received for publication 15 January 1980

On examination the following day the patient appeared well. Her temperature was normal and no pelvic mass could be found. However, Murphy's sign was still strongly positive. Her IUD was then removed; there was no growth of aerobic or anaerobic bacteria on culture. Urine culture for bacteria gave a negative result. Serum urea, serum and urine amylases, serum electrolytes, and serum transaminases (on four consecutive days) were all within normal limits. Cultures of stool specimens on three consecutive days gave negative results for yersinia, salmonellae, and shigellae. Blood culture for bacteria gave repeatedly negative results. Culture of the culdocentesis aspirate yielded a *Peptostreptococcus*, sensitive in vitro to penicillin and metronidazole but resistant to tetracycline; the fluid was not cultured for chlamydia. Gynaecological examination five days later was normal. The ESR was 25 mm in the first hour, WBC count, $5{\cdot}9\times 10^9$/l, and the IFAT titre for antichlamydial antibodies on serum obtained nine days after the first specimen had risen to 1024 (an eightfold increase). The patient was discharged on the tenth day after admission. Follow-up examination showed no abnormalities and the patient was asymptomatic.

Discussion

Except by laparoscopy, there is no specific test for diagnosing perihepatitis. Severe right upper abdominal pain and tenderness may be indistinguishable from acute cholecystitis.[8] It has been previously estimated that about 3% of patients with signs and symptoms of acute cholecystitis actually have gonococcal perihepatitis.[8] On the other hand, the incidence of gonococcal perihepatitis has been estimated to be less than 10% in women with gonococcal infection.[9] *C trachomatis* has been implicated as an important cause of non-specific genital infections, which are at present a major health problem.[10 11]

Recent results have provided strong support for the view that acute salpingitis may, in many cases, be caused by *C trachomatis*.[12-14] In this case, chlamydial infection was confirmed by positive culture results from cervical and urethral specimens. Furthermore, high antichlamydial antibody titres with a significant rise between the acute and convalescent phases suggested that *C trachomatis* actively participated in this systemic disease. Concomitant infection with *N gonorrhoeae* was definitely excluded by negative culture results. Further support for the aetiological role of *C trachomatis* was obtained from the poor response to the initial treatment with penicillin and metronidazole, both of which are known to be ineffective against chlamydia. However, when doxycycline treatment was started the patient rapidly responded clinically. Acute cholecystitis was an unlikely cause of the patient's symptoms as consecutive liver function tests showed normal results.

The fact that culture of the culdocentesis aspirate yielded *Peptostreptococcus* is interesting and raises the question of its possible aetiological role in the disease. Together with the modern techniques of anaerobic bacteriology, studies using culdocentesis have suggested that obligate anaerobes often play a part in the aetiology of acute PID.[15] However, caution should be used in interpreting the bacteriological results from specimens obtained by culdocentesis because of possible contamination of the specimens by micro-organisms from the vaginal flora.[16] In this case the *Peptostreptococcus* isolated was sensitive to penicillin and metronidazole in vitro, but this combined treatment was ineffective clinically, thus not supporting its aetiological role.

It is still uncertain how the infection reaches the peritoneum and the subphrenic space. A small amount of exudative fluid is not uncommon with PID and is thought to be produced by the inflamed tubal and ovarian tissue.[17] The precise aetiology of ascites, or overproduction of fluid within the peritoneal cavity, is difficult to establish. However, it may result from the rapid spread of a genital tract infection to the peritoneum, which—secondary to inflammation and adhesion formation—blocks the normal lymphatic pathways for excretion of fluid from the cavity.[18] Thus perihepatic inflammation is most likely caused by infection which has ascended from the pelvis.

In conclusion, *C trachomatis*, a prevalent sexually transmitted organism, may be a probable cause of exudative peritonitis and perihepatitis as a complication of acute PID. This syndrome has earlier been related only to gonococcal PID. This report may encourage clinicians to look for evidence of chlamydial infection in similar cases.

References

1. Litt IF, Cohen MI. Perihepatitis associated with salpingitis in adolescents. *JAMA* 1978;**240**:1253-4.
2. Semchyschyn S. Fitz-Hugh-Curtis syndrome. *J Reprod Med* 1979;**22**:45-8.
3. Curtis AH. A cause of adhesions in the right upper quadrant. *JAMA* 1930;**94**:1221-2.
4. Fitz-Hugh T Jr. Acute gonococcic peritonitis of the right upper quadrant in women. *JAMA* 1934;**102**:2094-6.
5. Müller-Schoop JW, Wang SP, Munzinger J, Schläpfer HU, Knoblauch M, Amman RW. *Chlamydia trachomatis* as a possible cause of peritonitis and perihepatitis in young women. *Br Med J* 1978;i:1022-4.
6. Paavonen J, Saikku P, Vesterinen E, Lehtovirta P. Infertility and cervical *Chlamydia trachomatis* infections. *Acta Obstet Gynecol Scand* 1979;**58**:301-3.

7. Saikku P, Paavonen J. Single-antigen immunofluorescence test for chlamydial antibodies. *J Clin Microbiol* 1978;**8:**119-21.
8. Knorring J, Nieminen J. Gonococcal perihepatitis in a surgical ward. *Ann Clin Res* 1979;**11:**66-70.
9. Stanley MM. Gonococcic peritonitis of the upper part of the abdomen in young women. *Arch Intern Med* 1946;**78:**1-13.
10. Anonymous. Non-specific genital infection. *Br Med J* 1979;ii:161-2.
11. Paavonen J. Chlamydial infections. Microbiological, clinical and diagnostic aspects. *Med Biol* 1979;**57:**135-51.
12. Mårdh P-A, Ripa T, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977;**296:**1377-9.
13. Treharne JD, Ripa KT, Mårdh P-A, Svensson L, Weström L, Darougar S. Antibodies to *Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis* 1979;**55:**26-9.
14. Paavonen J, Saikku P, Vesterinen E, Aho K. *Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis* 1979;**55:**203-6.
15. Eschenbach DA, Buchanan TM, Pollock HM, *et al.* Polymicrobial etiology of acute pelvic inflammatory disease. *N Engl J Med* 1975;**293:**166-71.
16. Cunningham FG, Hauth JC, Gilstrap LC, Herbert WNP, Kappus SS. The bacterial pathogenesis of acute pelvic inflammatory disease. *Obstet Gynecol* 1978;**52:**161-4.
17. Plentl AA, Friedman EA. *Lymphatic System of the Female Genitalia.* Philadelphia: WB Saunders Co, 1971.
18. Berek JS, Darney PD. Massive exudative ascites produced from a tubal pseudocyst in chronic pelvic inflammatory disease. *Obstet Gynecol* 1979;**54:**490-2.

Br J Vener Dis 1980;56:344-6.

A case of lymphogranuloma venereum of 20 years' duration

Isolation of Chlamydia trachomatis from perianal lesions

MICHAEL DAN,* HESCHI H ROTMENSCH,† EMMANUEL EYLAN,‡
ARDON RUBINSTEIN,† RAFAEL GINSBERG,§ AND MEIR LIRON†

*From the Departments of *Internal Medicine D and †Internal Medicine E, Municipal-Governmental Medical Centre; the ‡Department of Human Microbiology, Tel-Aviv University Sackler School of Medicine; and the §A Felix Public Health Laboratory, Ministry of Health, Tel-Aviv, Israel*

SUMMARY *Chlamydia trachomatis* was isolated from perianal lesions in a patient in whom lymphogranuloma venereum had been diagnosed 20 years previously. Treatment with tetracycline resulted in a pronounced improvement. Although *C trachomatis* is known to persist for a long time in the host, isolation of the micro-organism from infected tissues after such a prolonged period has not previously been reported.

Introduction

Published reports on chlamydial infections show an increasing interest in the subject. In addition to its long established role in trachoma, inclusion conjunctivitis, and lymphogranuloma venereum (LGV), the aetiological role of *Chlamydia trachomatis* in a growing number of diseases has been documented.[1] Little is known however about the natural history of chlamydial infection. Although LGV has occurred less frequently in the past decades, we describe here a case of perianal LGV of 20 years' duration in a male patient.

Case report

An 80-year-old man was admitted to hospital in December 1978 because of polypoid perianal lesions accompanied by constipation and perianal discharge. The perianal findings had been first noted 20 years previously when the presenting symptom was pruritus ani; the abundant purulent discharge compelled the patient to use several dressings a day. A biopsy performed on these lesions in 1961 showed multiple granulomas with diamond-shaped suppurating centres and multinucleated giant cells of foreign-body type characteristic of LGV (fig 1). The patient received no specific therapy at that time.

Clinical examination in 1978 showed perianal polypoid lesions, some of which were fluctuant, masking the anal orifice. Several perianal fistulas produced purulent discharge (fig 2). The remainder of the examination, including the genital and the inguinal areas, was unremarkable. Routine laboratory tests showed a moderately increased erythrocyte sedimentation rate (37 mm in first hour), and the presence of antinuclear factor (ANF).

Cultures of the anal discharge gave negative results, including those for *Mycobacterium tuberculosis* and fungi. Two serum samples gave a positive complement-fixation test result at a 1/32 dilution with a suspension of *Chlamydia psittaci* (Ornithosis Antigen, Behring Institute, Marburg/Lahn, W Germany). Serological tests (Wassermann, VDRL, Rappoport) for syphilis gave negative results. Chest roentgenogram, and small bowel and barium enema series, showed no abnormal findings. Proctoscopy demonstrated slightly hyperaemic mucosa in the anus but was otherwise normal. Histological sections of biopsy specimens showed chronic inflammation without granulomas or neoplastic changes. Perianal biopsy material was inoculated into McCoy cell cultures treated with cytochalasin B;[2] after 72 hours' incubation at 35°C typical chlamydial inclusions were found. Isolation was confirmed by passage of the organism in a second McCoy cell culture. Giemsa staining was performed for microscopical identification of the inclusion bodies specific for chlamydia (fig 3). Iodine staining gave a positive result for glycogen indicating *C trachomatis*. Further

Address for reprints: Dr H H Rotmensch, Department of Internal Medicine E, Rokach Hospital, PO Box 51, Tel Aviv, Israel

Received for publication 14 November 1979

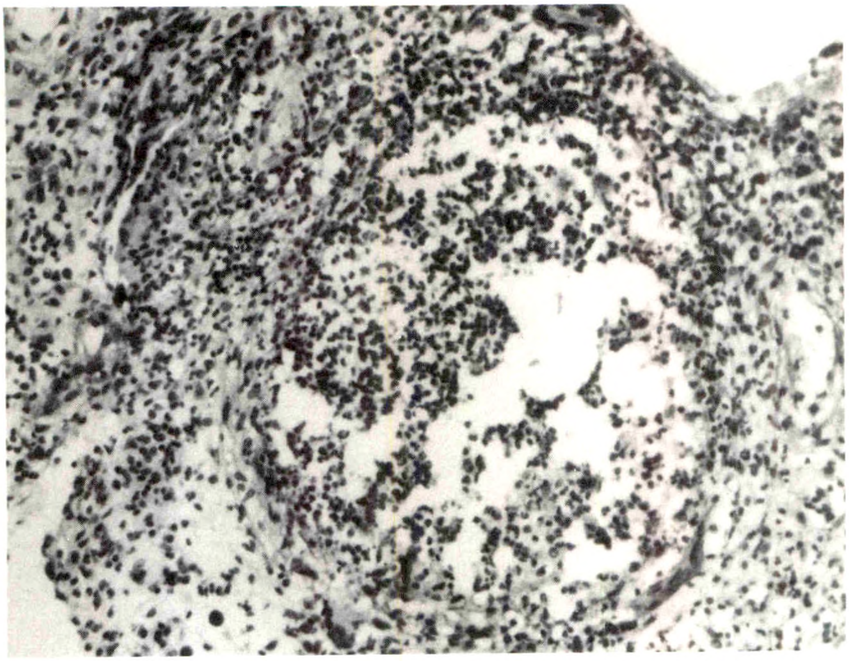


FIG 1 *Anal biopsy performed in 1961 showing a granuloma with suppuration (hematoxylin and eosin, magnification × 250).*

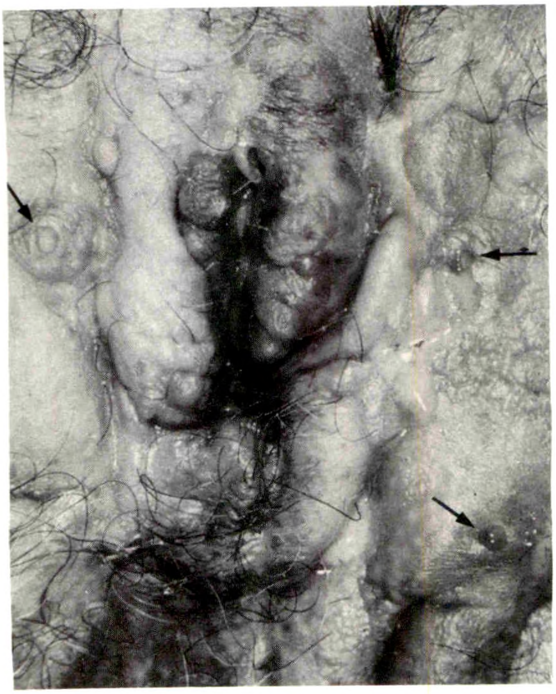


FIG 2 *Polypoid lesions of the perianal region with fistulas discharging pus (indicated by arrows).*

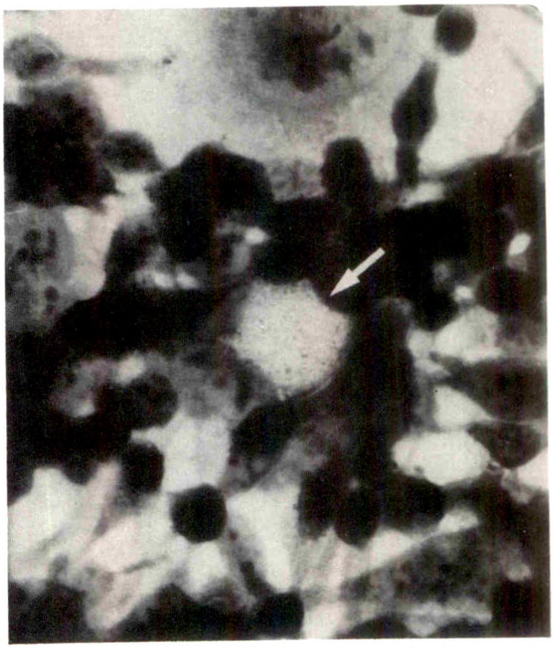


FIG 3 *Chlamydial inclusion bodies in McCoy tissue cultures as indicated by arrow (Giemsa staining, magnification × 1000).*

laboratory investigations showed normal serum immunoglobulin and complement concentrations but a marked suppression of cell-mediated responses to phytohaemagglutinin, concanavalin A, and pokeweed mitogens.

After LGV had been diagnosed, the patient was given oral tetracycline 2 g per day for 30 days. This resulted in a marked reduction of the anal discharge and disappearance of constipation and rectal pain.

Comment

LGV usually spreads during sexual intercourse through contact with infected material from primary lesions or ruptured lymph nodes and with anal discharge. Homosexuality has been considered important in the aetiology of anorectal lesions in men.[3] A non-venereal transmission by way of clothing has also been reported.[4] Our patient gave no history of homosexual contact.

Several authors[5 6] have stated that the agent of LGV can persist in the human host for many years. Recently, *C trachomatis* was reisolated from the genital tract of asymptomatic women after 15 months' follow-up.[1] However, isolation of this intracellular micro-organism after such a long period, as in the present case, has not been previously documented.

Various laboratory findings have been reported in cases of LGV, such as hyperglobulinaemia, autoimmune serum factors, and increased erythrocyte sedimentation rate.[4 7] In our patient the only positive laboratory findings were the presence of ANF and a moderately increased erythrocyte sedimentation rate.

To the best of our knowledge, no information is available about the immunological status of patients with LGV. In the present case, normal concentrations of immunoglobulins and complement were found while cell-mediated immunity was markedly impaired. It remains unclear whether this impairment was the underlying factor in the persistence of the chlamydial infection or whether the cellular immune defect can be attributed to immunosuppression as a result of chronic infection.

The authors are indebted to Drs A Behar, S Shibolet, and E Rubinstein for their contribution in the preparation of the manuscript.

References

1. McCormack WM, Alpert S, McComb DE, *et al.* Fifteen-month follow-up study of women infected with *Chlamydia trachomatis. New Engl J Med* 1979;**300:**123-5.
2. Sompolinsky D, Richmond S. Growth of *Chlamydia trachomatis* in McCoy cells treated with cytochalasin B. *Appl Microbiol* 1974;**28:**912-4.
3. Greaves AB. The frequency of lymphogranuloma venereum in persons with perirectal abscesses, fistulas in ano, or both. *Bull WHO* 1963;**29:**797-801.
4. Becker LE. Lymphogranuloma venereum. *Int J Dermatol* 1976;**15:**26-33.
5. Saad EA, de Gouveia OF, da Silva JK. Treatment of lymphogranuloma venereum of the anus and colon with glucocorticoids. *Am J Trop Med Hyg* 1962;**11:**108-14.
6. Schachter J. Chlamydial infections. *New Engl J Med* 1978;**298:**428-35.
7. Sonck CE, Räsänen JA, Mustakallio KE, *et al.* Autoimmune serum factors in active and inactive lymphogranuloma venereum. *Br J Vener Dis* 1973;**49:**67-8.

Br J Vener Dis 1980;**56**:347-8.

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Amoebiasis and giardiasis in New York City: a response

Sir

Kean and his co-workers, in their paper entitled "Epidemic of amoebiasis and giardiasis in a biased population" (1979; 56:375-8)[1] attempted to demonstrate on the basis of the study of an admittedly highly biased group of 126 individuals that a dangerous epidemic of amoebiasis and giardiasis presently exists in New York City. They also state that the public health authorities of New York City have so far only shown a token interest in this subject. We would like to reply to this biased statement.

A paper that purports to be a scientific study and then draws far-reaching epidemiological conclusions on the basis of a very small and highly biased population seems to us to be more in the nature of an editorial judgment than an addition to scientific knowledge. Kean *et al's* study adds nothing to our present knowledge. It has been obvious for some years that there are many individuals in New York City infected with amoebiasis and the total number of reported cases of this disease has been rising steadily in New York City each year. It is not unreasonable to assume that many cases go unreported. The disease is almost certainly more prevalent in the sexually active gay male community.

This was known to public health authorities before the publication of this paper by Kean *et al*. Indeed, the New York City Health Department has taken numerous steps to notify private physicians in New York City, as well as the gay community, of the situation during the past few years. Several articles by members of this department have appeared in local and national medical journals, beginning in 1977, to alert physicians to this problem. The first issue of the New York City Health Department's *Sexually Transmitted Disease Newsletter*, which Kean *et al* mistakenly implied was the only attempt on the part of the Health Department to publicise this problem, was devoted to the subject of enteric sexually transmitted diseases in the gay male community. This newsletter was sent to over 2000 selected practising physicians in New York City, especially those with large numbers of patients with sexually transmitted diseases. In addition, a press release was then issued by the New York City Health Department, which led to articles being written on the subject of amoebiasis and giardiasis in the many newspapers and publications widely read by the gay population (*Soho News* and others) as well as in more traditional newspapers such as *The New York Times*. As far back as 1977 the Bureau of Venereal Disease Control of the New York City Health Department had already developed an information booklet on amoebiasis and giardiasis for distribution to gay patients and others who are at high risk of contracting these diseases; this was cited by the Center for Disease Control in Atlanta for its excellence and is the only publication in colour available on the subject. This booklet has been widely distributed all over the United States as well as to many high-risk individuals in New York City. Numerous meetings were held at the Bureau of Venereal Disease Control of New York City Health Department with physicians who see large numbers of homosexual patients to alert them to the magnitude of this problem. Studies were conducted by the Bureau of Venereal Disease Control of the New York City Health Department to elucidate the situation further. Some of these studies have already been published[2-6] and others will be published in the near future.[7] A letter concerning this problem written by the director of the Bureau was published in the county medical society bulletin in 1978. Another letter was sent to all chiefs of service of hospitals and medical schools in the City in 1979. The success of these efforts can be seen by the fact that in 1978, for the first time, the National Institute of Allergy and Infectious Diseases included parasitic diseases, such as amoebiasis and giardiasis, in its applications for grant funds for research on sexually transmitted diseases.

We believe it is perfectly proper for Kean *et al* to call for more to be done in this area. Certainly, we would support greater federal involvement, especially in expanding the congressional mandate for Venereal Disease Control by the United States Public Health Service to include sexually transmitted enteric protozoal infections as well as many other sexually transmitted diseases not presently included. However, we strongly disagree with the implied statement that the situation that presently exists in New York City threatens to cause a massive epidemic, with numerous deaths, of the type that occurred in 1933. Medicine has advanced a great deal since that time. Effective treatments are available for amoebiasis and giardiasis which were not available in 1933. It is only too easy to attempt to galvanise the public health authorities of this country to fight every new menace to health that seems to appear over the horizon. As we learned from the swine influenza epidemic, such massive interventions do not always lead to improved public health.

In conclusion, we believe that the prevalence of amoebiasis and giardiasis in sexually active homosexual men in New York City at the present time represents a public health problem of great importance. However, this problem will not be solved by an admittedly biased paper and histrionic heuristics evoking memories of the pre-antibiotic era. It can be solved by co-operative efforts on the part of the medical and gay communities. We are initiating an effort of this type in New York City at the present time. Indeed, a new tropical disease clinic is being built in the Department of Health's district health centre in New York's largest gay neighbourhood. This new clinic will replace two older ones recently closed down—because of New York City's financial crisis—in areas where amoebiasis and giardiasis are far less prevalent than they are in Chelsea and Greenwich Village. This clinic will be in the same building as New York City's largest STD clinic and will probably be the only place in the world where a patient with STD can be investigated and treated for both syphilis and sexually transmitted amoebiasis in the same centre.

If Dr Kean is so concerned about this "epidemic" why did he not contact either or both of us, or for that matter the Commissioner of Health, when he "realised" his findings? We are also puzzled by his apparent failure to report his cases to the Health Department, a requirement of the New York City Health Code.

Yours faithfully,
Yehudi M Felman
John S Marr

Bureau of Venereal Disease Control,
93 Worth Street (Room 806),
New York,
NY 10013,
USA

References

1. Kean BH, William DC, Luminais SK. Epidemic of amoebiasis and giardiasis in a biased population. *Br J Vener Dis* 1979; **55**:375-8.
2. William DC, Felman YM, Marr JS, *et al.* Sexually transmitted enteric pathogens in the homosexual population. *NY State J Med* 1977;**77**:2050-2.
3. Felman YM, William DC. Sexually transmitted amebiasis. *Medical Aspects of Human Sexuality* 1978;**12**:155-6.
4. William DC, Shookhoff HB, Felman YM, *et al.* High rates of enteric protozoal infections in selected homosexual men attending a venereal disease clinic. *Sex Transm Dis* 1978;**5**:155-7.
5. Felman YM, Riccardi NB. Sexually transmitted enteric diseases. *Bull NY Acad Med* 1979;**55**:533-9.
6. Felman YM, Nikitas JA. Sexually transmitted enteric pathogens in New York City. *NY State J Med* 1979;**79**:1412-3.
7. Pomerantz BM, Marr JS, Goldman WD. Amebiasis in New York City 1958-1978; Identification of the male homosexual high-risk population. *Bull NY Acad Med* 1980 (in press).

Notices

Wellcome Museum

The Wellcome Museum of the History of Medicine is now housed at the Science Museum in South Kensington, where it will form the basis of a national medical collection. Two new galleries will open there in 1980 and 1981 to provide a permanent display of medical history.

It has been drawn to our attention that material relating to the history of venereology and venereal diseases is scattered, fragmentary, and often in danger of being destroyed. This is particularly the case for the twentieth century, during which time venereology has been reorganised as a distinct branch of medicine, with a result that records are not necessarily held centrally.

The materials of interest might range from three dimensional objects such as instruments, literature such as printed forms or leaflets, photographs, and manuscript notes (ephemeral or otherwise), all of which can supply valuable background information for the study of venereology and the work of the clinics.

The historian to the collection will be very glad to hear from readers with any items of this kind: Dr Christopher Lawrence, The Wellcome Museum of the History of Medicine, Science Museum, South Kensington, London SW7 2DD (telephone 01-589-3456, ext 565).

RSM Meeting: "Sexually Transmitted Diseases"

A meeting of the Section of Epidemiology and Community Medicine of the Royal Society of Medicine is to be held on Thursday 13 November 1980 at 5.30 p.m.

The speakers—all from the Academic Department of Genitourinary Medicine at the Middlesex Hospital, London—will be: Professor M W Adler (on "The size of the problem—how accurate?"); Miss E M Belsey (on "Cases versus patients—multiple attenders identified"); and Mr R M Harrison (on "The role of symptoms in treatment seeking patterns of women with genital symptoms").

Those intending to attend should apply to the Sections Office of the Royal Society of Medicine, 1 Wimpole Street, London W1M 8AE, for the appropriate attendance voucher.

Bulletin of sexually transmitted diseases

The Dutch Foundation against Venereal Diseases is now publishing a quarterly bulletin, *Bulletin Sexueel Overdraagbare Aandoeningen.* Further information can be obtained from Dr W T A M Henderikx, John F Kennedylaan 99, 3981 G B Bunnik, Postbus 100, 3980 C C Bunnik, the Netherlands.

Correction

In the letter by P B Carey and J N Johnson (1980;**56**:57) on "Diverticular disease presenting at a special clinic" the first reference should have read, ". . . *Br J Urol* 1969;**41**:314-9."

Obituaries

JAMES MARSHALL (1913-79)

After 31 years in South Africa, James Marshall collapsed and died on 12 November 1979 aged 66 years, for 15 of which he had been the distinguished head of the department of dermatology at the Medical School of the University of Stellenbosch. Earlier in 1948, when he started a private practice in Johannesburg, he was given charge of the department of skin diseases at the University of Pretoria and, with a natural linguistic bent, was soon able to lecture in Afrikaans, which he continued to do after moving to the Cape in 1956. Apart from his longstanding international reputation, to quote a colleague, "The credit for the high standard of dermatology in South Africa goes to him in no small measure."

It has also been written that Marshall arrived in dermatology "through the doorway of venereal diseases" and his passage will be remembered with much affection by older venereologists, especially those associated with him in London during the war years.

Born in Glasgow in 1913, after qualifying with distinction at the London Hospital at the young age of 21, he later served as resident medical officer at the London Lock Hospital. Having studied for a while in Paris (perfecting his already able French tongue) he acquired a life-long addiction for that country's medical and culinary traditions. (His visits to Europe later in life were centred more on France than Britain.)

Come the war, he joined the Army to be posted as venereologist to the Military Isolation Hospital, Harrow Road, London, previously the female section of the Lock Hospital, which catered only for skin and venereal diseases. Here, as major, he wrote his textbook on *The Venereal Diseases,* which was published in 1944. Subsequently (as lieutenant colonel) he succeeded Brigadier T E Osmond as adviser in venereology to the War Office, which post he held until the end of 1945, by which time he had initiated a private practice based in Jermyn Street, London.

Back in civilian life he was soon appointed director of the venereal disease clinic at the Royal Northern Hospital and then consulting dermatologist to the Central Middlesex County Hospital. Almost immediately his companion book, *The Skin Diseases,* in which he promulgated his belief along with the French school of dermatologists "that dermatology and syphilology are inseparable," was published in 1948. By the time the second edition of *The Venereal Diseases* appeared also in the same year—the National Health Service was then being introduced in Britain—his decision to emigrate to South Africa had already been taken.

His down-to-earth approach to life—abundantly laced with wit and humour, tinged with cynicism, but tempered with a warmth and generosity—gave him a magnetic quality which endeared him to his friends. He certainly showed the writer wider vistas, particularly the full enjoyment which can be obtained from venereology not least from combining routine work with research and writing.

He was happily married to Eileen Mary Platt for 40 years, to whom the deepest sympathies are extended.—RRW.

SAJJAD HAMEED QURESHI (1937-80)

Dr "Sam" Qureshi, who died recently under tragic circumstances at the age of 42, was undoubtedly the best registrar I have been associated with in my clinics, and he became a firm friend. His medical career was a distinguished one and among his many earlier achievements was the award of "best graduate of the year" in 1958 at the King Edward Medical College (Punjab).

After a series of varied junior posts in hospitals in this country he became neurological registrar at the Churchill Hospital, Oxford, and was in virtual charge of the tubercular meningitis unit where he could exercise his undoubted medical skill to the full. After a spell of four years as registrar at the STD clinic at the Central Middlesex Hospital he obtained the post of senior registrar at the STD clinic, St Thomas's Hospital. He was clearly ready to become a prominent consultant in his field when his illness forced him to become a patient at that hospital, where he died showing exemplary courage during this tragic period.

The first and lasting impressions I formed were a sense of calm, compassion, competence, and a sense of humour. This made him deservedly popular with colleagues and patients alike. He was also exceptional in many fields, being chosen for the Olympic hockey team to represent his country, but he declined in order to devote his time and energy to medicine, which remained his lasting love. He was very well read, outstandingly musical, and an excellent organiser.

In have no doubt that our profession has lost a splendid consultant-to-be and the best way I can sum up his many good qualitities is by saying that he was a gentleman in the literal sense. He will be missed by his many friends and faithful patients and they will extend their genuine sympathy to his wife.—GC

Br J Vener Dis 1980;**56**:350-4.

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
  (Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea
  (Clinical; microbiology; therapy)
Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases
Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (clinical and therapy)

Syphilis of the rectum mimicking neoplastic disease
O VOINCHET AND M GUIVARC'H (Suresnes, France). *Gastroenterol Clin Biol* 1980; 4:134-8.

Syphilis (serology and biological false-positive phenomenon)

Improvement of syphilis screening in blood donors by TPHA test
A KLUGE, E BORZANI, AND D ROELCKE (University of Heidelberg, West Germany). *Blut* 1980;**40**:117-22.

Syphilis (pathology and experimental)

The effect of reducing and other agents on the motility of *Treponema pallidum* in an acellular culture medium
WSK CHALMERS AND D TAYLOR-ROBINSON (Clinical Research Centre, Harrow, UK). *J Gen Microbiol* 1979; **114**:443-7.

This very interesting paper reports the results of investigations on the effects of glutathione, thioglycollate, cysteine, and dithiothreitol on the survival of *Treponema pallidum* in an acellular medium. These agents are used to maintain the redox potential (E_h) of −275±25mV at pH 7·3, which has been found necessary for the survival of the organism. A medium based on that required for organ cultures and differing considerably from that given for the TPI test[1] was used.

The organisms were grown in a gas mixture containing 92% N_2, 5% CO_2, and 3% O_2. *T pallidum* (Nichols strain) was provided by the Venereal Disease Reference Laboratory, London. New Zealand white rabbits were inoculated intratesticularly, and seven days later the treponemes were extracted from the orchitic testes by shaking in the medium. Counts of treponemes were performed using a Thoma-Weber darkground chamber and survival times for 50% (ST_{50}) and 10% (ST_{10}) motile treponemes determined.

The effect of the addition of fetal calf serum (FCS) and bovine serum albumin (BSA) to the basic medium containing all four reducing agents was studied. It was found that FCS 20% and BSA 0·5% increased the ST_{50} from 11 to 52 hours and the ST_{10} from 21 to 98 hours.

The reducing agents were selectively omitted from the basic medium containing 10% FCS and 0·5% BSA. Thioglycollate was found to be more toxic than glutathione, but when both were omitted and the E_h maintained with cysteine and dithiothreitol the ST_{50} increased from 16 to 122 hours and the ST_{10} from 80 to 163 hours. In this medium FCS at 20% had no obvious advantage over FCS at 10%.

G D Morrison

Reference

1. Wilkinson AE. Serological tests for syphilis. In: Wilkinson AE, Taylor CED, McSwiggan DA, Turner GC, Rycroft JA, Lowe GH, eds. *Laboratory Diagnosis of Venereal Disease*. London: HMSO. 1972:13-5.

Effect of *Treponema pallidum*-infected testes supernatants on the cellular response of normal rabbit lymphocytes
K WICHER, V WICHER, AND D KAMINSKI (New York State Department of Health, USA). *Infect Immun* 1980;**27**:846-50.

Gonorrhoea (clinical)

Perihepatitis gonorrhoeica. Fitz-Hugh-Curtis syndrome
E SUNDAL AND GA STALDER (Kantonsspital, Basel, Switzerland). *Schweiz Med Wochenschr* 1980; **110**:540-1.

Gonorrhoea (microbiology)

Natural serum bactericidal activity against *Neisseria gonorrhoeae* isolates from disseminated, locally invasive and uncomplicated diseases
PA RICE, WM McCORMACK, AND DL KASPER (University of Boston, USA). *J Immunol* 1980; **124**:2105-9.

Samples of serum from 10 healthy volunteers were tested for bacteriocidal activity against strains of gonococci from 22 patients with disseminated gonorrhoea (DGI), 13 women with uncomplicated gonorrhoea (UC), and 18 women with pelvic inflammatory disease (PID). Strains from patients with only dermatitis and arthralgia were significantly more resistant than strains from patients with either UC or PID, while strains from patients with suppurative arthritis were more sensitive to the bacteriocidal activity of normal sera. Similarly, strains from patients with severe

PID were more sensitive than those from patients with mild or moderate disease. Patients with suppurative arthritis developed bacteriocidal antibodies more consistently than those with only dermatitis and arthralgia. PID and DGI strains associated with bacteriocidal antibodies in their hosts (largely those with more severe disease) were also more sensitive to sera from volunteers.

The authors suggest that the bacteriocidal activity they detected may be an important predictor of the specific interaction of gonococci with the human host.

Brian Evans

Comparative evaluation of the tube and microdilution limulus lysate techniques for rapid presumptive diagnosis of gonococcal urethritis in men

RB PRIOR AND VA SPAGNA (Ohio State University, USA). *J Clin Microbiol* 1980; **11:** 340-2.

Eighty-one men with urethritis were investigated. Serial dilutions (ranging from 1/100 to 1/102 400) of urethral exudates were examined by the standard tube and microdilution limulus amoebocyte lysate methods and the results obtained compared with those of Gram staining and culture. The accuracy of predicting the culture results was 98% for a dilution of 1/200 and 99% for a dilution of 1/400 for the tube and microdilution methods respectively. It was concluded that the microdilution limulus method was a reliable, rapid, and sensitive diagnostic aid.

A McMillan

Evaluation of rapid carbohydrate degradation tests for identification of pathogenic *Neisseria*

DJ PIZZUTO AND JA WASHINGTON (Mayo Clinic, Rochester, USA). *J Clin Microbiol* 1980; **11:** 394-7.

A total of 156 clinical isolates were tested by the modified rapid fermentation test, the BACTEC *Neisseria* differentiation kit, and the cystine-trypticase agar method. The modified rapid fermentation test and the BACTEC methods accurately identified at least 95% of 101 strains of *N gonorrhoeae* tested and at least 91% of 45 strains of *N meningitidis* tested within four hours. Overall the cystine-trypticase agar method was the most accurate (97%) but required as long as 48 hours' incubation. The data presented appear to show that rapid carbohydrate degradation tests can provide reliable and specific identification results.

Authors' summary

The diagnostic value of the gonococcal complement fixation test

H YOUNG, C HENRICHSEN, AND A McMILLAN (University of Edinburgh, UK). *Med Lab Sci* 1980; **37:** 165-70.

To assess the diagnostic value of a gonococcal complement fixation test (GCFT) with *Neisseria gonorrhoeae* strain 9 as antigen as a routine screening procedure, sera from female patients attending a sexually transmitted diseases clinic were investigated. The test gave a positive result in 35% of women with culture-positive gonorrhoea; the performance of the test was noted to be directly related to the duration of infection, the positivity rate being 70% when the infection had been present for two weeks or more.

Of 297 patients attending the clinic but in whom there was no clinical, epidemiological, laboratory, or presumptive evidence of gonococcal infection past or present, 7% gave an unexplained positive result. The test was reactive in 1% of control patients attending the antenatal clinic; although there was no clinical or epidemiological evidence of gonorrhoea, these patients were not investigated fully.

The role of the GCFT in the diagnosis of gonorrhoea is discussed.

Authors' summary

Confirmatory identification of *Neisseria gonorrhoeae* by slide coagglutination

DV LIM AND T WALL (University of South Florida, USA). *Can J Microbiol* 1980; **26:** 218-22.

Polygenes and modifier genes for tetracycline and penicillin resistance in *Neisseria gonorrhoeae*

PF WARNER, EJ ZUBRZYCKI, AND M CHILA (Temple University, Philadelphia, USA). *J Gen Microbiol* 1980; **117:** 103-10.

Characterization of serologically dominant outer membrane proteins of *Neisseria gonorrhoeae*

RL McDADE AND KH JOHNSTON (University of Texas, USA). *J Bacteriol* 1980; **141:** 1183-91.

Comparison of virulence markers of peritoneal and Fallopian tube isolates with endocervical *Neisseria gonorrhoeae* isolates from women with acute salpingitis

DL DRAPER, JF JAMES, GF BROOKS, AND RL SWEET (University of California, USA). *Infect Immun* 1980; **27:** 882-8.

Antigenic diversity of the serotype antigen complex of *Neisseria gonorrhoeae:* analysis by an indirect enzyme-linked immunoassay

KH JOHNSTON (University of Texas, USA). *Infect Immun* 1980; **28:** 101-10.

Immunogenicity of ribosomal preparations from *Neisseria gonorrhoeae*

MD COOPER, RP TEWARI, AND DV BOWSER (University of South Illinois, USA). *Infect Immun* 1980; **28:** 92-100.

WSJM, a simple chemically defined medium for growth of *Neisseria gonorrhoeae*

TP WONG, RK SHOCKLEY, AND KH JOHNSTON (University of Texas, USA). *J Clin Microbiol* 1980; **11:** 363-9.

Interaction of lectins with *Neisseria gonorrhoeae*

PZ ALLEN, MC CONNELLY, AND MA APICELLA (University of Rochester, USA). *Can J Microbiol* 1980; **26:** 468-74.

Increasing resistance of *Neisseria gonorrhoeae* to penicillin and co-trimoxazole: an in-vitro study

RA BHUJWALA, RN PANDHI, OP SINGH, AND S SHRINIWAS (All India Institute of Medical Sciences, New Delhi). *Ind J Med Res* 1980; **71:** 501-4.

In-vitro antimicrobial activity of cefoperazone, cefotaxime, moxalactam (LY 127935), azlocillin, mezlocillin, and other β-lactam antibiotics against *Neisseria gonorrhoeae* and *Haemophilus influenzae* including β-lactamase-producing strains

CN BAKER, C THORNSBERRY, AND RN JONES (Centre for Disease Control, Atlanta, USA). *Antimicrob Agents Chemother* 1980; **17:** 757-61.

On the question of chromosomal gene transfer via conjugation in *Neisseria gonorrhoeae*
VI STEINBERG AND ID GOLDBERG (University of Kansas, USA). *J Bacteriol* 1980; **142:** 350-4.

Gonorrhoea (therapy)

Sulfamethoxazole-trimethoprim synergism against *Neisseria gonorrhoeae*
MF REIN, WC ELLIOTT, JM SWENSON, AND C THORNSBERRY (University of Virginia, USA). *Antimicrob Agents Chemother* 1980; **17:** 247-50.

Spectinomycin therapy for gonorrhea in prepubertal children
PJ RETTIG, JD NELSON, AND H KUSMIESZ (University of Texas, USA). *Am J Dis Child* 1980; **134:** 359-63.

Non-specific genital infection

The effect of cortisol on glycogen and fructose-1, 6-diphosphatase in baby hamster kidney cells infected with *Chlamydia trachomatis*
SI REED AND WD HANN (Bowling Green State University, Ohio, USA). *Can J Microbiol* 1980; **26:** 135-40.

Antimicrobial drug susceptibility of 15 strains of *Chlamydia trachomatis* recently isolated from cases of non-gonococcal urethritis in Italy (letter)
R CEVENINI, MP LANDINI, M DONATI, AND F RUMPIANESI (University of Bologna, Italy). *J Antimicrob Chemother* 1980; **6:** 294-6.

Isolation and purification of a type-specific antigen from *Chlamydia trachomatis* propagated in cell culture utilizing molecular shift chromatography
JT HOURIHAN, TR ROTA, AND AB MacDONALD (Harvard University, Massachusetts, USA). *J Immunol* 1980; **124:** 2399-405.

A mouse model of *Chlamydia trachomatis* pneumonitis
C-C KUO AND W-J CHEN (University of Washington, USA). *J Infect Dis* 1980; **141:** 198-202.

Experimental salpingitis in grivet monkeys by *Chlamydia trachomatis:* modes of spread of infection to the Fallopian tubes
BR MØLLER AND P-MÅRDH (Aarhus University, Denmark). *Acta Pathol Microbiol Scand* 1980; **88:** 107-14.

Suppression of *Chlamydia trachomatis* inclusion formation by fetal calf serum in cycloheximide-treated McCoy cells
RT EVANS (Clinical Research Centre, Harrow, UK). *J Clin Microbiol* 1980; **11:** 424-5.

The genital mycoplasmas (first of two parts)
D TAYLOR-ROBINSON AND WM McCORMACK (Clinical Research Centre, Harrow, UK). *N Engl J Med* 1980; **302:** 1003-10.

The genital mycoplasmas (second of two parts)
D TAYLOR-ROBINSON AND WM McCORMACK (Clinical Research Centre, Harrow, UK). *N Engl J Med* 1980; **302:** 1063-7.

Reiter's disease

Reiter's disease in women
DL SMITH, RM BENNETT, AND MG REGAN (Providence Medical Centre, Portland, USA). *Arthritis Rheum* 1980; **23:** 335-40.

Twenty-nine patients with seronegative arthritis were screened over four years for additional clinical features associated with Reiter's disease. Their age range was 13-71 years. A history of sexually transmitted disease and dysentery was not included.

Arthritic involvement was oligoarticular in 14 patients, polyarticular in 14, and monarticular in one. Nineteen patients complained of sacro-iliac tenderness although radiographic changes were found in only five. Enthesopathic lesions were found as Achilles tendinitis or plantar fasciitis in 15 patients, and tenosynovitis was found as characteristic digital tendon involvement (sausage-shaped digits) in 20 patients.

Seventeen patients were HLA-B27-positive but there did not appear to be an association with increasing severity of disease.

The difficulties found in diagnosing Reiter's disease were acknowledged. The incomplete presentation of the classical triad of arthritis, urethritis, and conjunctivitis was recognised in association with duration of the disease. Of the 29 patients, 21 experienced chronic arthritis, five had a "relapsing" arthritis, and three had an acute arthritis of short duration.

The other diagnostic features were ocular involvement in 10 patients, urinary tract involvement in 10 patients, nail changes in five, and keratoderma blennorrhagica in five. Urinary investigations were limited to clinical history and observation of pyuria and haematuria. The cervix was not assessed. Two of the five patients with keratoderma had pyuria, but a skin biopsy specimen from one of the patients showed changes histologically indistinguishable from pustular psoriasis.

Treatment was effected with indomethacin and phenylbutazone in most patients with control of symptoms and improvement of arthritis in 19 patients. One patient responded to intramuscular gold and another patient with extensive teratoderma improved with 6-mercaptopurine.

J M Harvey

Candidosis

Efficacy of econazole in the treatment of candidiasis and other vaginal discharges
A RUBIN, JM RUSSELL, AND A MAUFF (Baragwanath Hospital, Johannesburg, South Africa). *S Afr Med J* 1980; **57:** 407-8.

Inhibitors of sterol biosynthesis in *Candida albicans* by imidazole-containing antifungals
MS MARRIOTT (Pfizer Centre for Research, UK) *J Gen Microb* 1980; **117:** 253-5.

Alteration of polymorphonuclear leukocyte activity by viable *Candida albicans*
AE HILGER AND DL DANLEY (University of Chicago, USA). *Infect Immun* 1980; 714-20.

High incidence of sensitivity to yeast killer toxins among *Candida* and *Torulopsis* isolates of human origin
EJ MIDDELBECK, JMH HERMANS, C STUMM, AND HL MUYTJENS (Catholic University of Nijmegen, Netherlands). *Antimicrob Agents Chemother* 1980; **17:** 350-4.

Trichomoniasis

Trichomonas vaginalis: re-evaluation of its clinical presentation and laboratory diagnosis

AC FOUTS AND SJ KRAUS (Center for Disease Control, Atlanta, USA). *J Infect Dis* 1980; **141:** 137-43.

From data relating to 400 women with vaginitis who attended a sexually transmitted disease clinic it was shown that a purulent frothy discharge is characteristic of trichomonal vaginitis. However, if this is the sole diagnostic criterion, 88% of infected women will not be identified and 29% of non-trichomonal vaginitis will be erroneously diagnosed. The authors found that culture of vaginal exudate on a modified Feinberg-Whittington medium was twice as sensitive as wet-mount microscopy in demonstrating infection. The well known association of gonococcal infection and trichomoniasis was again demonstrated.

A McMillan

A comparison of five methods for the detection of *Trichomonas vaginalis* in clinical specimens

PN LEVETT (University of Surrey, UK). *Med Lab Sci* 1980; **37:** 85-8.

Penetration of metronidazole into preformed cavities in rabbits

T HOFSTAD AND K SVEEN (University of Bergen, Norway). *J Antimicrob Chemother* 1980; **6:** 275-8.

No cytogenetic effect of metronidazole (letter)

F MITELMAN, B STROMBECK, AND WB URSING (Lund University Hospital, Sweden). *Lancet* 1980; i: 1249-50.

Genital herpes

In-vitro and in-vivo resistance of herpes simplex virus to 9-(2-hydroxyethoxymethyl) guanine (Acycloguanosine)

KO SMITH, WL KENNELL, RH POIRIER, AND FT LYND (University of Texas, USA). *Antimicrob Agents Chemother* 1980; **17:** 144-50.

Human T-cell response to herpes simplex virus antigen in vitro

EJ BERLE AND E THORSBY (National Hospital of Norway, Oslo). *Acta Pathol Microbiol Scand* 1980; **88:** 31-8.

Tubular structures in the cervix of mice experimentally infected with herpes simplex virus type 2

T IWASAKA, H ODA, Y HINO, AND R MORI (University of Kyushu, Japan). *J Med Virol* 1980; **16:** 117-22.

Other sexually transmitted diseases

Serological testing for hepatitis B in male homosexuals: special emphasis on hepatitis B e antigen and antibody by radioimmunoassay

RL MURPHY, MT SCHREEDER, JE MAYNARD, SC HADLER, AND MJ SHELLER (Centre for Disease Control, Phoenix, USA). *J Clin Microbiol* 1980; **11:** 301-3.

The importance of sexual activity in transmission of hepatitis B virus (HBV) is well recognised. In homosexual males hepatitis B surface antigen and antibody (anti-HBs) rates of 5% and 50% respectively have been noted. This paper describes results of specimens sent from five clinics (two attended exclusively by homosexuals and three municipal venereal disease clinics). Specimens were tested for HBsAg and anti-HBs at initial bleeding and at four months. If specimens gave negative results for HBsAg and anti-HBs they were tested for antibody to hepatitis B core antigen (anti-HBc). Specimens with positive results for HBsAg, anti-HBs, and anti-HBc were examined for hepatitis B e antigen (HBeAg) and its antibody (anti-HBe).

Of 3785 persons tested 234 (6·2%) had detectable HBsAg, 1990 (52·5%) had positive results for anti-HBs without detectable HBsAg, and 126 (3·3%) had positive results for anti-HBc without HBsAg or anti-HBs. The combined HBV seropositivity rate (for HBsAg or anti-HBs or anti-HBc) was 62%. Of 572 specimens with positive results for HBsAg on initial determination 217 were examined for HBeAg and anti-HBe by radioimmunoassay (RIA). Of these, 147 (67·7%) specimens were HBeAg-positive and 50 (23%) were anti-HBe-positive.

Results for HBeAg and anti-HBe were found by RIA in 105 specimens from individuals who were initially HBV-seronegative but were HBsAg-positive at follow up at four months. Of those who had HBsAg, 29 (64·4%) were HBeAg-positive and six (13·3%) were anti-HBe-positive. An evaluation of individuals who were HBsAg-positive at the initial determination indicated that 198 (91%) of 217 had HBeAg or anti-HBe detectable by RIA.

Serological markers for hepatitis B virus in male homosexuals demonstrated a high prevalence of past and present infection.

Michael Waugh

Hepatitis B serology in Greek prostitutes: significance of the different serum markers

E KAKLAMANI, A KYSIAKIDOU, D TRICHOPOULOS, G PUPONTSAKIS, I KOMMANDAKI, AND D KARALIS (University of Athens, Greece). *J Hyg (Camb)* 1980; **84:** 257-61.

The frequency and significance of hepatitis B surface antigen (HBsAg), its antibody (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc) were studied in 198 prostitutes and compared with 117 female controls of low socioeconomic class.

Serological evidence of previous viral hepatitis B (HBV) infection was found in 97% of the prostitutes compared with 45% among the controls. The proportion of HBsAg carriers among those previously infected by HBV was the same in the two groups indicating that co-factors, which may affect the establishment or persistence of the HBsAg carrier state, are equally distributed among prostitutes and controls. A high prevalence of anti-HBc alone was found in prostitutes (21%) and controls (47%) previously infected. Further quoted surveys have suggested that the presence of anti-HBc alone indicates either active infection or resolution from the chronic carrier state. In view of these findings it was concluded that anti-HBc alone may also be indicative of past infection. A high prevalence of coexisting anti-HBc and anti-HBs antibodies was found in prostitutes (75%) compared with controls (38%) with evidence of previous infection. This was thought to indicate continuous exposure to HBV favouring frequent and repeated boosters for both antibody types.

R S Pattman

Amebiasis in New York City 1958-1978: identification of the male homosexual high-risk population

BM POMERANTZ, JS MARR, AND WD GOLDMAN (Suny Downstate Medical Center, New York, USA). *Bull NY Acad Med* 1980;**56:**232-44.

In 1978, 1875 cases of amoebiasis were notified in New York City. This compares with 1307 cases of tuberculosis, 1260 of hepatitis, and 20[illegible]0 of primary and secondary syphilis. There has been an increased awareness among male homosexuals that they are at risk to this disease. This has been coupled with an increased awareness among physicians serving the group. Laboratory diagnostic facilities have improved at the same time.

It became obvious to the New York Department of Health that there was a need for identification of high-risk areas. Prevalence data were therefore noted for New York City. The highest areas of attack rate were Lower West Side, 154·3 per 100 000, and Kip's Bay (Upper East Side), 130·1 per 100 000. It was noted in 1978 that in Manhattan 71% of notified cases were male compared with 53% outside that area. In Lower West Side the male-to-female ratio was 4·8:1. In one part of Lower West Side, south of 14th Street and west of 4th Avenue, the rate was 543·9 per 100 000 men (25 times the overall rate). Furthermore, 82% of cases were notified from one private laboratory.

The data collected suggested that cases of amoebiasis were more frequent in identifiable areas of New York City, that there was a correlation in increased incidence of amoebiasis in homosexual males who indulged in frequent oro-anal sexual activities, and that there was some spread to women by bisexual males and by homosexual food handlers.

M A Waugh

Non-condylomatous cervical wart virus infection

R REID, CR LAVERTY, M COPPLESON, W ISARANGKUL, AND E HILLS (Wayne State University, USA). *Obstet Gynecol* 1980; **55:**476-83.

A histopathological study of cervical human papillomavirus (HPV) infection was carried out over six months. To define this lesion clearly cytological smear samples were taken from 7281 patients and colposcopically directed punch biopsy specimens were taken from 183 patients.

The distinction was made between the more prominent, fungating, keratinised vulval condylomas and the subclinical, flat, cervical, epithelial proliferation, which the authors call non-condylomatous cervical wart virus infection (NCWVI).

Demonstration of virus particles was achieved in 24 out of 53 cases submitted to transmission electron microscopy (TEM), either from the target biopsy specimens or from the exfoliative cells in the original cytological smears.

But for the availability of TEM in detecting the virus particles, the histological and cytological findings of NCWVI could cause some difficulty in distinguishing it from straightforward cervical intraepithelial neoplasia (CIN). However, the study has also presented some characteristic features which could be used in diagnosis. These include the presence in NCWVI of cells with a striking perinuclear space called either koilocytotic, halo, or balloon cells. The dense peripheral cytoplasm of these cells, seen on histology or exfoliated in cytology, takes up several stains producing an unusual orange or purple colour.

CIN always occurs within the metaplastic epithelium area but NCWVI affects both this transformation zone and the original squamous ectocervical and vaginal epithelium. This feature is also picked out when using acetic acid, and whereas CIN lesions have a dull appearance and are flat NCWVI is shiny and shows keratosis. Colposcopic contour provides further distinction for NCWVI ranging from a micropapilliferous pattern to a "gyrus and sulcus" pattern.

In CIN vascular atypia seen on colposcopy includes the classic punctation and mosaic within sharply demarcated areas, whereas in NCWVI the patterns are poorly formed with less defined borders but occasional capillary loops.

The authors conclude the discussion by making three points: firstly, that NCWVI and CIN must sometimes coexist; secondly, that recurrence of CIN after treatment should not exclude diagnosis of NCWVI instead; and, thirdly, that perhaps the association between HPV and CIN is causal although not so far proved.

J M Harvey

Miscellaneous

Effect of trimethoprim and trimethoprim-sulfamethoxazole on development of drug-resistant vaginal and fecal floras

SJ PANCOAST, DM HYMANS, AND HC NEU (Colombia University, New York, USA). *Antimicrob Agents Chemother* 1980; **17:** 263-8.

Metronidazole for non-specific vaginitis (letter)

M BURDI, G MANENTI, D MATTIONI, AND L LASALA (Ospedale Civile, Balneario, Italy). *Lancet* 1980; i: 1029.

Quantification and significance of mast cells in lesions of Behçet's disease

C LICHTIG, S HAIM, I HAMMEL, AND R FRIEDMANBIRNBAUM (Rambam Medical Centre, Haifa, Israel). *Br J Dermatol* 1980; **102:**255-9.

A clinical comparative study of multiple sclerosis and neuro-Behçet's syndrome.

S MOTOMURA, T TABIRA, AND Y KUROIWA (University of Kitskyushu, Japan). *J Neurol Neurosurg Psychiatry* 1980; **43:** 210-3.

Inner ear involvement in Behçet's disease

I BRAMA AND M FAINARU (Hebrew University, Jerusalem, Israel). *Arch Otolaryngol* 1980; **106:**215-7.

FORTY-EIGHT HOUR PATIENCE

One tablet nightly for three nights means that your patient's patience only needs to last 48 hours. That's all it takes to clear vaginal candidiasis – twice as conveniently as the old One Two Free.

Canesten®

clotrimazole

New 200mg vaginal tablet now only one a night for three nights

Presentations Canesten is available either as 200 mg vaginal tablets in packs of three with applicator or as 100 mg vaginal tablets in packs of six with applicator. Canesten Duopak contains six 100 mg vaginal tablets with applicator plus 20 g cream containing 1.0% clotrimazole. **Dosage** *Vaginal Tablets 100mg* Two vaginal tablets should be inserted at night for three consecutive days. Alternatively, one vaginal tablet daily for six days. *Vaginal Tablets 200mg* One vaginal tablet should be inserted at night for three consecutive days. *Duopak* Vaginal Tablets 100 mg as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to prevent re-infection. **Side effects** Rarely patients may experience transient local mild burning or irritation. **Basic NHS Costs** 6 x 100 mg vaginal tablets with applicator £2.13; 3 x 200 mg vaginal tablets with applicator £1.99; Duopak £3.47. **Product Licence Numbers** Vaginal tablets 100 mg, PL 0010/0015; vaginal tablets 200 mg, PL 0010/0072; Duopak, PL 0010/0016 & PL 0010/0015. Further information is available from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. ® Registered Trade Mark of Bayer Germany.

M.S.S.V.D.

The Medical Society for the Study of Venereal Diseases

11 Chandos Street, Cavendish Square, London W1M 0EB

President: Dr. C. B. S. Schofield

The object of the Society is to bring together those interested or engaged in the diagnosis, treatment, and public health aspects of venereal diseases. The Society meets regularly in London for the reading of papers, discussions, and presentation of cases. An out-of-London meeting is held in the Spring.

Annual subscription for membership of the Society is £12 or $22.00 for all members.

Applications for membership are very welcome and forms can be obtained from the Honorary Secretary, Dr. J. Barrow, Lydia Department, St. Thomas' Hospital, SE1 7EH.

Programme 1980-81

Friday October 31st	Short papers	
Friday November 28th	The T.P.I. test	Dr. A. E. Wilkinson Dr. J. B. Selkon Professor J. Thivolet Dr. J. L. Fluker
Friday 30th January, 1981	Intestinal Parasites	Professor Lewis M. Drusin Dr. A. McMillan Dr. J. W. Ackers
Friday 27th March, 1981	Vulval Disease	Professor Charles Douglas
Friday 24th April, 1981	Presidential Address Higher Medical Training in Venereology	

TODAY'S TREATMENT/3

Diseases of the respiratory and urinary systems account for a high proportion of all consultations in general practice, justifying the 200 pages devoted to them in the third volume of Today's Treatment. The remaining section deals with the use of antibiotics across the whole spectrum of medicine, looking at the advantages and drawbacks of the major categories and their use in clinical settings where decision-making may be difficult. Like its predecessors, Today's Treatment/3 concentrates on practical issues and provides the busy clinician with the information he needs in his day to day practice.

Demy Octavo—Paperback—318 pages—ISBN 0 7279 0036 6

Price: Inland £5·50; Abroad US$13.75, including postage

(Concessionary price to BMA Members: Inland £5·00; Abroad US$12.50. When ordering BMA Members must quote their membership number or the full price will be applicable.)

Payment must be enclosed with order or a surcharge of 50p will be made for rendering invoices and statements.

Order your copy now from
The Publisher, BRITISH MEDICAL JOURNAL, BMA House,
Tavistock Square, London WC1H 9JR *or through any leading bookseller*

The combination of simple language and straightforward line drawings has made the

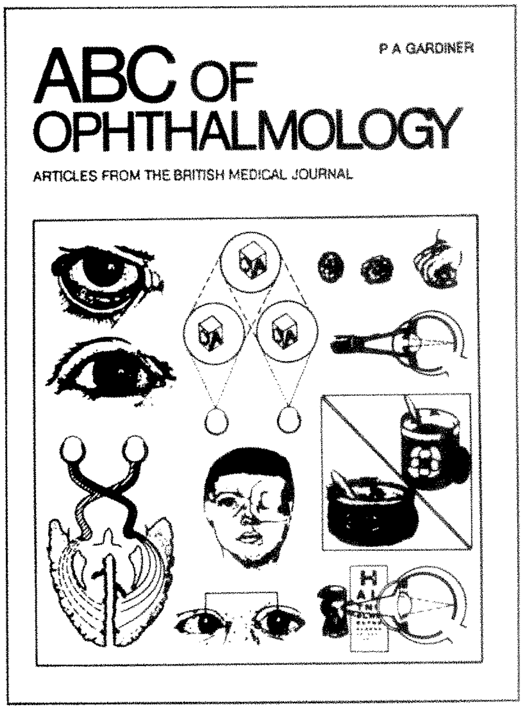
ABC OF OPHTHALMOLOGY

one of the most popular series of articles in the Medical Practice section of the British Medical Journal.

All the common problems are covered - visual defects, squints, glaucoma and cataracts - together with detailed instruction on examination, diagnosis and management.

The articles have now been collected into book form - in exactly the same format as they appeared in the BMJ - price: Inland £2.50; Overseas US$6.25 (Inland £2.00; Overseas US$5.00 for BMA members).

Order your copy now
From: The Publisher,
British Medical Journal,
BMA House,
Tavistock Square,
London WC1H 9JR
or any leading bookseller



P A GARDINER

ABC OF OPHTHALMOLOGY

ARTICLES FROM THE BRITISH MEDICAL JOURNAL

Reduced Subscription Rates for Junior Hospital Doctors Residents and Interns...

British Journal of Venereal Diseases

is available to junior hospital doctors in all parts of the world for a maximum of four consecutive years at REDUCED Annual Subscription Rates. (Thereafter, or when reaching consultant status this special offer will no longer apply.) The following reduced rates are unchanged for the six issues published in 1980.

Inland £13.00

Overseas US$30.00

(The full rates are: Inland £23.00; Overseas US$55.00)

The opportunity to subscribe to this journal of international authority and repute at a substantially reduced subscription rate is one not to be missed by junior doctors seeking to specialise in venereology.

Please complete the adjacent order form and send for your subscription NOW.

Further order forms can be supplied on request.

ORDER FORM

BMA Publications
BMA House, Tavistock Square
London WC1H 9JR, England

Please enter my subscription to

BRITISH JOURNAL OF VENEREAL DISEASES

☐ United Kingdom and Irish Republic £13.00

☐ Overseas US$30.00

☐ My remittance for .... is enclosed

(Please tick appropriate boxes)

NAME ....

....

ADDRESS ....

....

....

To be completed by Consultant

I hereby confirm that the above mentioned doctor is a member of the junior hospital staff/resident/intern at ....

....

Signed ....

BRITISH JOURNAL OF VENEREAL DISEASES

Sexually Transmitted Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $25.00 outside the United States ($22.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $28.00 in the United States and $30.00 in all other countries.

William O. Harrison, M.D.
American Venereal Disease Association
P.O. Box 22349
San Diego, California 92122 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $25.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases*.
☐ I wish to enter a Non-Member or institutional subscription ($28.00 U.S., all other countries $30.00).
(Mail non-member subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105)

Name ______________________________

____

Address ______________________________

City ______________ State Country ______________ Zip ______________

British Journal of Venereal Diseases October 1980 Vol. 56 No. 5

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Venereal diseases in the Pacific Islands: Papua New Guinea
R R WILLCOX *page* 277

Sexually transmitted diseases in Indonesia
MARWALI HARAHAP *page* 282

Mechanisms of selective toxicity of metronidazole and other nitroimidazole drugs
DAVID I EDWARDS *page* 285

Quantitative microhaemagglutination assay for *Treponema pallidum* antibodies in experimental syphilis
ROBERT R TIGHT AND ARTHUR C WHITE *page* 291

Autolymphocytotoxins in syphilis
N H L de JONG, J A M KOEHORST, J J van der SLUIS, AND A M BOER *page* 297

Concentric rings simulating tinea imbricata in secondary syphilis
P A SAROJINI, A D DHARMARATNAM, K PAVITHRAN, AND C GANGADHARAN *page* 302

Enzymatic detection of *Neisseria gonorrhoeae*
MILTON M TAKEGUCHI, HOWARD H WEETALL, DAVID K SMITH, HUGH C McDONALD, KIM A LIVSEY, CLARENCE C DETAR, AND THOMAS A CHAPEL *page* 304

Sensitivity to penicillin of gonococci in different racial groups
P RODIN, A D SETH, D M KING, AND A E WILKINSON *page* 308

Comparison of methods for the detection of penicillinase-producing *Neisseria gonorrhoeae*
E H SNG, K L YEO, V S RAJAN, AND A L LIM *page* 311

Vaginal chemoprophylaxis in the reduction of reinfection in women with gonorrhoea: clinical evaluation of the effectiveness of a vaginal contraceptive
CLIFFORD H COLE, THOMAS G LACHER, JAMES C BAILEY, AND DIANE L FAIRCLOUGH *page* 314

Diagnosis and management of anorectal gonorrhoea in women
VALERIE A STANSFIELD *page* 319

Oropharyngeal flora and individual susceptibility to neisserial infection
H YOUNG, A B HARRIS, AND D H H ROBERTSON *page* 322

Isolation of *Neisseria lactamica* from the female genital tract
WILLIAM A TELFER BRUNTON, HUGH YOUNG, AND DONALD R K FRASER *page* 325

Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in Greenland: a seroepidemiological study
P-A MÅRDH, I LIND, E FROM, AND A-L ANDERSEN *page* 327

Tetracycline in nongonococcal urethritis: comparison of 2 g and 1 g daily for seven days
WILLIAM R BOWIE, JOHN S YU, ARCHANA FAWCETT, AND HUGH D JONES *page* 332

Subclinical pneumonia due to serotypes D-K of *Chlamydia trachomatis*: case reports of two infants
ERIC M C DUNLOP, ROGER J HARRIS, SOHRAB DAROUGAR, JOHN D TREHARNE, AND SAFWAT S AL-EGAILY *page* 337

Chlamydia trachomatis as a possible cause of peritonitis and perihepatitis in a young woman
J PAAVONEN AND V V VALTONEN *page* 341

A case of lymphogranuloma venereum of 20 years' duration: isolation of *Chlamydia trachomatis* from perianal lesions
MICHAEL DAN, HESCHI H ROTMENSCH, EMMANUEL EYLAN, ARDON RUBINSTEIN, RAFAEL GINSBERG, AND MEIR LIRON *page* 344

Correspondence *page* 347 Correction *page* 348

Notices *page* 348 Obituaries *page* 349

Abstracts *page* 350

ASTM CODEN: BJVDAK 56 (5)277-354 (1980) ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

British Journal of
Venereal Diseases

UNIVERSITY OF MICHIGAN LIBRARY

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

A E WILKINSON
(Editor)
B A EVANS
(Assistant Editor)
A McMILLAN
(Assistant Editor, Abstracts)
C B S SCHOFIELD
(President, MSSVD)

J BARROW
(Hon Sec, MSSVD)
M W ADLER
G CAUSSE
G CSONKA
L M DRUSIN

J M KNOX
G D MORRISON
I PHILLIPS
E STOLZ
R N THIN
J WALLIN

EDITOR,
British Medical Journal

P D SIMMONS
(Indexer)
SHUNA JUDGE
(Technical Editor)

This *Journal*, founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A E Wilkinson, 22 Kingsend, Ruislip, Middlesex HA4 7DA. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals*, available from the Publishing Manager, *British Medical Journal*, BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; **55**: 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS. *Venereal Diseases*. 3rd ed. London: Baillière and Tindall, 1975.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977: 214-22.

PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £27.00 in the United Kingdom and Republic of Ireland and US$65.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US$65.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal*, 1172 Commonwealth Avenue, Boston, Mass. 02134. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases*, BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1980 by the *British Journal of Venereal Diseases*. This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.

Ecostatin™

econazole nitrate

"Once the infection has been established in the female, it can undoubtedly be transmitted to the male consort… This may, in turn… serve as a reservoir for the reinfection of his partner after her condition had responded successfully to treatment. The risk of this sexual 'ping-pong' has been recognised…"[1]

ECOSTATIN*
Pessaries: Each contain 150 mg econazole nitrate.
Twin Pack: Three pessaries plus 15 g Ecostatin cream. 1% w/w econazole nitrate.

Indications
Pessaries and Twin Pack: Vulvovaginal candidosis.

Actions
Econazole nitrate is a broad-spectrum antifungal agent.

Dosage and Administration
Pessaries and Twin Pack: One pessary to be inserted at bedtime for three consecutive nights. The cream to be applied twice daily to the anogenital area.

Precautions
The pessaries should not be used during the first trimester of pregnancy unless deemed essential for the patient's wellbeing.
Topical preparations should not be used in or near the eyes and used with care in the presence of eczematous dermatitis.

Side-effects
Occasional local irritation. The pessaries may cause transitory discomfort.

the most active fungicide available prevents 'sexual ping-pong'

Package quantities, prices and Product Licence Numbers
Pessaries: Pack of 3 with applicator – £3.60
PL No: 0085/0013
Twin Pack: 3 pessaries, applicator and 15 g cream – £4.70
—
Cream: 15 g – £1.25; 30 g – £2.50
PL No. 0085/0014

Product Licence Holder
F.A.I.R. Laboratories Ltd.,
179 Heath Road,
Twickenham, TW1 4BJ

(1) Br. J. Sex. Med. 1974, 1, 21.

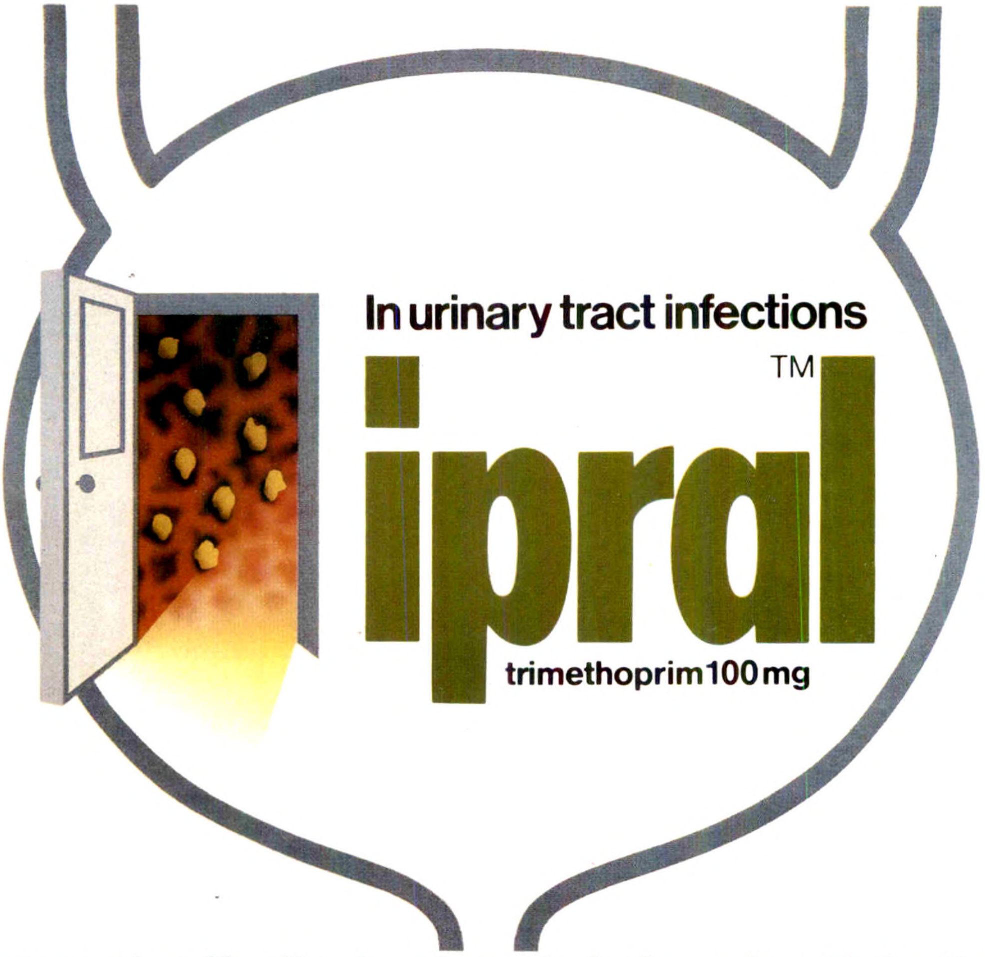
Just one pessary a night for three nights clears candidal vaginitis. The cream treats the anogenital region, and its simultaneous use by the male partner will prevent re-infection.

- **Total pessary disintegration independent of vaginal secretion.**
- **Ecostatin pessaries melt and disperse within ten minutes extensively coating the vaginal wall.**
- **Short course of treatment and acceptable presentation leads to improved patient compliance.**
- **Primary resistance has not been observed.**
- **Twin pack helps prevent re-infection and can be used to treat the sexual partner.**

Further information is available from
FAIR Laboratories Ltd., 179 Heath Road, Twickenham, Middx. TW1 4BJ

**Ecostatin is a registered trademark and FAIR Laboratories is the authorised user.*

LIBRARY



...the way to effective treatment of urinary tract infections

Trimethoprim – proved effective as a single entity without the side-effects associated with sulphonamides.

"Trimethoprim alone may well prove as effective as trimethoprim-sulphamethoxazole combinations ..."[1]

96% success in general practice.[2] Half as many side-effects.

Results of 107 Courses of Treatment in 96 Domiciliary Patients

Antibiotic	Overall Success
Ampicillin	89%
Cephalexin	62%
Co-trimoxazole	81%
Trimethoprim	**96%**

ipral simple treatment for acute infections – 2 tablets b.d.

ipral for routine use in chronic U.T.I. – 1 tablet b.d.

In a study on recurrent U.T.I.'s
"... trimethoprim is the most effective of the antibacterial agents examined."[3]

Greater efficacy with fewer side effects.

In the trial in acute U.T.I.'s

"... the total side effects caused by ampicillin, cephalexin and co-trimoxazole were similar and occurred twice as often as when trimethoprim was given alone ..."[2]

Antibiotic	No. of Patients	% Side Effects
Ampicillin	88	22
Cephalexin	84	25
Co-trimoxazole	83	21
Trimethoprim	**84**	**8**
Total	339	

Prescribing information

IPRAL tablets

Each tablet contains trimethoprim B.P. 100 mg.

Indications

Ipral is indicated for the treatment and prophylaxis of acute and chronic urinary tract infections.

Actions

Ipral is active in-vitro against most pathogenic Gram-positive and Gram-negative bacteria. Exceptions include Neisseria species and Pseudomonas aeruginosa.

Dosage and administration

For acute urinary tract infections 2 tablets twice daily is recommended. For long term and prophylactic therapy 1 tablet twice daily is recommended.

Contra-indications

Ipral should not be given to patients with severe renal insufficiency where blood levels cannot be monitored regularly. Ipral should not be used in pregnancy or during the first few weeks of life.

Precautions

Care is necessary in administration to patients with impaired renal function. Special precaution should be exercised in patients with a predisposition to folate deficiency.

Side-effects

Nausea, vomiting and skin rashes may occur but are rare. Ipral may depress haematopoiesis during prolonged therapy. This has proved reversible when treated with calcium folinate.

Treatment of overdosage

Gastric lavage. Calcium folinate may be used to counteract any effect of Ipral on bone-marrow.

Package quantities and price

Packs of 100 tablets £5.30.

Product Licence No. - 0034/0190.

Full prescribing information available from:
Technical Services Department, E.R. Squibb & Sons Ltd.
Squibb House, 141-149 Staines Road, Hounslow TW3 3JA.
Tel: 01-572 7422

Ipral is a Trade Mark of E. R. Squibb & Sons Ltd

...the way to effective treatment of urinary tract infections

- As effective as trimethoprim/sulphamethoxazole combination
- Avoids the side effects of sulphonamides
- At a sensible price

and "Trimethoprim given alone was shown to be much better tolerated."[2]

"Treatment with TMP (trimethoprim) alone is superior to the combined regimen due to a smaller incidence of side-effects; ..." thus "... introduction of TMP alone on the market is recommended."[4]

"Less gastrointestinal side-effects and skin rashes have occurred with trimethoprim ..."[3]

References
1. Lancet 1979, **1**, 493. 2. Br. med. J. 1972, **2**, 673.
3. Ann. clin. Res. 1978, **10**, (suppl. 22). 4. Chemother. 1973, **19**, 314.

ipral™

trimethoprim 100 mg

more than half the story

SQUIBB™ Full prescribing information available from: Technical Services Department, E.R. Squibb & Sons Ltd, Squibb House, 141-149 Staines Road, Hounslow TW3 3JA. Tel: 01-572 7422

MINOCIN

Minocycline
Hydrochloride
100 mg

TABLETS

for the treatment
of gonorrhoea and
non-specific urethritis[1-4]

1 Brit. J. Vener. Dis. (1975), 51, 97–98
2 Brit. J. Vener. Dis. (1975), 51, 206–209
3 Brit. J. Vener. Dis. (1976), 52, 43–45
4 Brit. J. Vener. Dis. (1978), 54, 107–111

INFORMATION ON THE USE OF MINOCIN
IN THE TREATMENT OF VENEREAL DISEASE
AND PRODUCT DATA WILL BE SUPPLIED
UPON REQUEST

Lederle Laboratories
Research for British Medicine
A division of Cyanamid of Great Britain Ltd
Fareham Road, Gosport, Hants PO13 0AS

MINOCIN is a trademark for minocycline hydrochloride

Br J Vener Dis 1980;**56**:355-62

Kinetic study of serum penicillin concentrations after single doses of benzathine and benethamine penicillins in young and old people

P COLLART, M POITEVIN, A MILOVANOVIC, A HERLIN, AND J DUREL
From the Alfred Fournier Institute, Paris, France

SUMMARY In a comparative kinetic study of the serum concentrations of two penicillin complexes—medium-long-acting (benethamine penicillin) and long-acting (benzathine bipenicillin)—after a single injection in young adults and elderly people, the following results were confirmed statistically: (a) age was a major factor in the variations in serum penicillin concentrations and in their persistence in the serum; (b) the penicillin was absorbed faster in young than in elderly subjects even when a long-acting complex was used; (c) serum concentrations below the level regarded as lethal for treponemes appeared much earlier and more frequently in young than in old people; and (d) the bioequivalence between penicillin preparations could not be estimated solely from the number of units of the agent used but from the bioavailability of the chosen formulation.

Thus a uniform and standard penicillin dosage allowing no safety margin may help in the superficial healing of a syphilitic chancre or the resolution of a roseola but it will certainly be insufficient to kill *Treponema pallidum.* It seems essential therefore to provide an antibiotic cover at high dosage over a long period of time.

Introduction

Penicillin has long been the drug of choice for the treatment of syphilis,[1] [2] and many penicillin preparations have been made available.[3] However, in the absence of properly compiled statistics[4] over an adequate period to assess the results of therapy, many specialists have tended to base the dosage solely on the number of international units (IU).[2]

It would be interesting, without attempting to carry out an exhaustive study on the kinetics of excretion of medium-acting and long-acting combinations of penicillin containing either benethamine penicillin[5-7] or benzathine bipenicillin,[8-10] to compare their bioavailability after an intramuscular injection of a single standard dose, the choice of dosages being based on generally acceptable standards for the treatment of syphilis.[2 9 11-17]

At present there are many factors capable of affecting the elimination of penicillin. The influence of age on the bioavailability of these products was chosen for this study; in fact, age modifies many physiological functions but its influence on the kinetics of drugs is as yet almost unknown.[18]

Investigations have been performed on penicillins with a short action.[19-23] No comparative study however has been carried out on delayed-acting penicillins to demonstrate (a) the degree to which a drug becomes available to the systemic circulation and the rate at which this occurs (bioavailability) in relation to age (that is, how the clinical effectiveness of a drug can be correlated with the measurements of its serum concentration after administration and the time-dose relationship)[24-26]; and (b) the influence of the pharmaceutical preparation on the intensity and the period of action of the agent in relation to age. To provide a better basis for the statistical analysis of our results, the values were weighted in terms of plasma volume using the nomogram of Dagher *et al*,[27] in which the weight, age, and sex of the subjects are considered.

The aim of this paper is not to propose a therapeutic plan but, considering the results obtained, to present data which will help doctors to choose and adjust doses for each individual patient in the treatment of syphilis.[28]

Address for reprints: Dr P Collart, Institut Alfred Fournier, 25 Boulevard Saint-Jacques, 75014 Paris, France

Received for publication 3 October 1978

Subjects and methods

CHOICE OF SUBJECTS

Four groups of healthy subjects were selected for the study[29]:

Group 1a, consisting of young adults (20 male, mean age 20·5±1·2 years, mean weight 63·8±6·5 kg) given benethamine penicillin complex;

Group 1b, consisting of young adults (14 male and one female, mean age 22±2·8 years, mean weight 64·8±6·5 kg) given benzathine bipenicillin complex;

Group 2a, consisting of elderly subjects (24 female and 12 male, mean age 75±8·4 years, mean weight 58·1±12·9 kg) given benethamine penicillin complex; and

Group 2b, consisting of elderly subjects (20 female and five male, mean age 76±9·3 years, mean weight 58·7±10·5 kg) given benzathine penicillin complex.

Renal function was systematically checked by measuring the creatinine clearance, which was normal.

The weight and urinary output of all subjects were recorded and investigations were carried out to ensure that none of them received any other therapy during the test period which might affect the serum penicillin concentrations.

CHOICE OF PENICILLIN

Two types of penicillin were selected:

(a) a complex with medium-long action, benethamine penicillin, composed of benethamine penicillin 600 000 IU and penicillin G sodium 400 000 IU; and

(b) a complex with prolonged action, benzathine bipenicillin, composed of benzathine benzylpenicillin 600 000 IU, penicillin G procaine 300 000 IU, and penicillin G sodium 300 000 IU.

Each of these products was injected by the deep intramuscular route in a single dose (that is, 3 million IU of the benethamine penicillin complex and 2·4 million IU of the benzathine bipenicillin complex diluted in 6 ml of solvent).

SAMPLING

As the long-term action of these complexes was the essential factor to be investigated, sampling began only after 48 hours.[30] All the blood samples were morning fasting specimens taken at the following intervals: (a) for the benethamine penicillin complex, after 2, 3, 4, 5, and 6 days (group 1a), and after 2, 3, 4, 5, 6, 13, and 20 days (group 2a); and (b) for the benzathine bipenicillin complex, after 2, 4, 6, 9, 11, and 13 days (group 1b), and after 2, 4, 6, 9, 11, 13, and 20 days (group 2b). Serum was decanted from the blood collected and the analyses performed during the following hour.

SERUM PENICILLIN MEASUREMENTS

Numerous techniques have been suggested for these measurements, as described by Chabbert[31] in France and Lightbown in Britain.[32] In this study, we used the method recommended by Bennet *et al*[33] based on the principle of gel diffusion of the penicillin from wells; this technique provides reproducible and accurate results (less than 10% error).

Preparation of plates

A sheet of plain glass measuring 400 × 400 × 8 mm was prepared by gluing glass rods along its edges; this gave an area sufficient for up to 81 measurements. The plate was covered with a sheet of perforated stainless steel, and the whole assembly was sterilised in dry heat.

Media

The media used were the Difco Bacto antibiotic media Nos 1 and 2, which have a pH of 6·6. They were poured in two layers to give a total thickness of 3·5 mm. The upper layer (medium No 1) of 1 mm contained the test micro-organism *Sarcina lutea* (ATCC 93 41) in exponential growth phase, which was adjusted with a spectrophotometer to a level of 10^6 bacteria/mm^3 ($\lambda = 650$ nm). The serum for analysis was introduced into depressions formed in the gel, which had a capacity of 150 μl.

Readings

The plate prepared as described was incubated for 22 hours at 30°C. The inhibition diameters were then read by reference to a standard range of penicillin obtained with a pool of undiluted human serum from normal subjects. A line of best fit determined by the least squares method was used to estimate the penicillin concentration.

STATISTICAL ANALYSIS

Statistical analysis was performed by the mean of the non-parametric tests of Friedmann and Mann-

TABLE I *Values of the coefficients A and B and r^2 for the graphic estimation of the variations in serum penicillin concentrations for each of the penicillin complexes and for each of the four groups of subjects*

		Serum penicillin concentrations (units/ml)		
Subject group	*Penicillin complex*	*Coefficient A*	*Coefficient B*	r^2
1a (Young)	Benethamine penicillin	0·184	−0·019	0·95
2a (Elderly)	Benethamine penicillin	0·229	−0·003	0·79
1b (Young)	Benzathine bipenicillin	0·208	−0·014	0·99
2b (Elderly)	Benzathine bipenicillin	0·510	−0·015	0·92

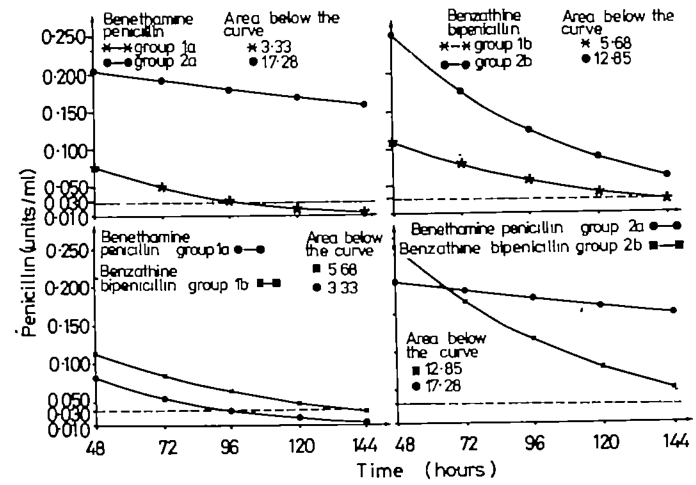


FIG 1 *Regression curves of serum penicillin concentrations after a single injection of benethamine penicillin or benzathine bipenicillin in four different groups (groups 1a and 1b, young subjects; groups 2a and 2b, elderly subjects). Results are in weighted values.*

Whitney.[30] [34-36] Friedmann's two-way analysis of variance confirmed that between the 48th and 144th hour the decrease in serum penicillin concentration was statistically significant within the same group. The model with the best fit of regression for the graphic estimation of the variations in serum penicillin concentration was the decay-type exponential function. The values of the coefficients and of r^2—that is, the proportion of total variation about the mean y explained by the regression—are presented in table I. (These coefficients were used to plot the curves shown in fig 1.)

THERAPEUTIC ASSESSMENT

As *T pallidum* is one of the few micro-organisms which has not yet been cultivated, most of the biological notions that we have about this spirochaete have been established only by comparison with other microbial elements, which are cultivatable in vitro or with what is observed in animal experiments.[37] [38]

Thus for penicillin to be effective in the treatment of syphilis, it was shown that the duration of the penicillinaemia should be maintained over an arbitrary minimum concentration,[1] [2] usually taken as 0·03 units of penicillin per ml of serum.[39-42] For the purpose of assessing the treponemicidal action of penicillin concentrations in serum, the lethal dose was assumed to be 0·03 IU/ml.

Results

BENETHAMINE PENICILLIN COMPLEX

Group 1a (young subjects)

After a single injection of benethamine penicillin 3 million IU in 20 young subjects (group 1a), the mean serum concentration was 0·247 units/ml 48 hours after injection, which is much higher than the minimum concentration regarded as treponemicidal (table II). However, 10% of these subjects had serum penicillin concentrations below this borderline (<0·03 units/ml)—that is, sublethal concentrations (fig 2). The mean concentration decreased quite rapidly in the following few days and was 0·035 units/ml on the sixth day. At that stage 40% of the group had serum penicillin concentrations of <0·03 units/ml (fig 2).

TABLE II *Comparison of serum penicillin concentrations after injections of benethamine penicillin and benzathine bipenicillin in four different groups of subjects*

Subject group	*No in group*	*Mean age (years)*	*Penicillin complex*	*Dose (IU)*	*Mean serum penicillin concentrations (units/ml) at:*						
					2 Days	*3 Days*	*4 Days*	*5 Days*	*6 Days*	*13 Days*	*20 Days*
1a (Young)	20	20·5	Benethamine penicillin	3×10^6	0·247	0·139	0·078	0·053	0·035		
2a (Elderly)	36	75	Benethamine penicillin	3×10^6	0·488	0·421	0·351	0·390	0·342	0·104	0·042
1b (Young)	15	22	Benzathine bipenicillin	$2{\cdot}4\times10^6$	0·340		0·161		0·080	0·037	
2b (Elderly)	25	76	Benzathine bipenicillin	$2{\cdot}4\times10^6$	0·635		0·216		0·151	0·081	0·064

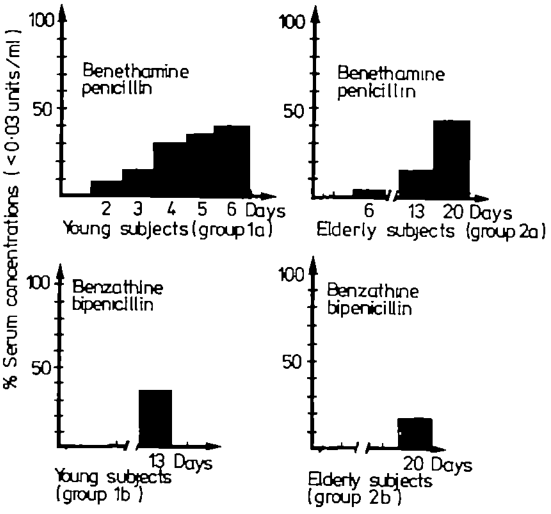


FIG 2 *Percentage of mean serum penicillin concentrations <0·03 units/ml in four groups of subjects.*

Group 2a (elderly subjects)

After the same dose of benethamine penicillin in 36 elderly subjects (group 2a) the mean serum concentration after 48 hours was 0·488 units/ml, which was much higher than the figure for group 1a; none of these subjects had a sublethal concentration. These concentrations also decreased but more slowly than in the young subjects; at 20 days after the injection the mean concentration was 0·042 units/ml. Nevertheless, by this time 43% of this older group had serum penicillin concentrations below the treponemicidal concentration (fig 2).

Statistical analysis

The statistical results are presented in table III and fig 1. For each time interval the serum penicillin concentrations were significantly higher ($P<0·0001$) in the elderly subjects (group 2a). Thus after 48 hours the mean concentrations were twice as high in elderly as in young subjects. The two curves then fell almost in parallel, although the serum concentrations in the elderly subjects remained well above the lethal concentrations for *T pallidum* and for a much longer period (20 days instead of six). Moreover, the proportion with a sublethal concentration (<0·03 units/ml) reached 40% in young subjects after six days but in elderly subjects only after 20 days.

BENZATHINE BIPENICILLIN COMPLEX

Group 1b (young subjects)

After a single injection of benzathine bipenicillin 2·4 million IU (that is, 600 000 IU less than the dose of benethamine penicillin) in 15 young subjects (group 1b), the mean serum penicillin concentration was 0·340 units/ml after 48 hours (table II). None of this group had a serum concentration <0·03 units/ml on

TABLE III *Variations in serum penicillin concentrations after injection of benethamine penicillin or benzathine bipenicillin in four groups of subjects**

	Serum penicillin concentrations (units/ml)† at (hours):		
Statistical comparisons	*48*	*96*	*144*
Benethamine penicillin			
Group 1a (young)	0·079±0·011	0·027±0·004	0·011±0·004
Group 2a (elderly)	0·210±0·028	0·166±0·021	0·159±0·034
Z‡	4·360	5·765	5·546
P	<0·0001	<0·0001	<0·0001
Benzathine bipenicillin			
Group 1b (young)	0·110±0·013	0·052±0·004	0·029±0·003
Group 2b (elderly)	0·281±0·038	0·097±0·012	0·068±0·008
Z‡	3·716	2·067	3·450
P	0·0001	0·02	0·0003
Groups 2a and 2b			
Benethamine penicillin	0·210±0·028	0·166±0·021	0·159±0·033
Benzathine bipenicillin	0·281±0·038	0·097±0·012	0·068±0·008
Z‡	1·410	2·579	3·053
P	NS	0·005	0·001
Groups 1a and 2a			
Benethamine penicillin	0·079±0·011	0·027±0·004	0·011±0·004
Benzathine bipenicillin	0·110±0·013	0·052±0·004	0·029±0·003
Z‡	2·283	3·383	3·534
P	0·01	0·0005	0·0002

*Results in weighted values
†Mean ± error of mean
‡Number of standard normal variates

the second day. On the thirteenth day the mean concentration was 0·037 units/ml but by that time 33% of the subjects had sublethal concentrations (fig 2).

Group 2b (elderly subjects)

After the same dose of benzathine bipenicillin in 25 elderly subjects (group 2b), the mean serum concentration was 0·635 units/ml after 48 hours—that is, twice the figure observed in the younger group (group 1b); none of these subjects had a concentration below the minimum concentration regarded as treponemicidal. On the other hand, the concentration then decreased much more rapidly until the twentieth day when the mean serum concentration was 0·064 units/ml and 20% of the subjects had a concentration <0·03 units/ml (table II and fig 2).

Statistical analysis

The statistical analyses of the mean weighted values are shown in table III and fig 1. These values indicated that the serum penicillin concentrations were significantly higher ($P<0·02$) in the elderly subjects (group 2b) but, unlike with benethamine penicillin, the values tended to converge rapidly. Thus, 48 hours after the injection the elderly subjects again had concentrations twice as high as the young subjects, but the serum penicillin curve fell much more quickly after that in the former group, although it consistently remained above the curve in young subjects on the thirteenth day but in only 20% of elderly subjects, at least under the conditions of our experiment.

COMPARISONS BETWEEN PENICILLIN COMPLEXES

Groups 1a and 1b (young subjects)

Although the dose of benethamine penicillin injected was 600 000 IU greater than the dose of benzathine bipenicillin (2·4 million IU), the mean concentrations found after 48 hours were lower for the former preparation than for the long-acting penicillin (table II, fig 3). The two curves then fell in parallel to reach the minimum treponemicidal limit after about five days with benethamine penicillin and after 13 days with benzathine bipenicillin. At these two points in time the percentages of sublethal concentrations were 33% for benzathine bipenicillin and 40% for benethamine penicillin (fig 2). The mean serum weighted concentrations and the calculated value of Ƶ (the number of standard normal variates) are given in table III and fig 1. These show that the mean serum concentrations for the benzathine bipenicillin complex were significantly higher between the 48th and 144th hours than those observed after injection of a single dose of 3 million units of the benethamine penicillin complex.

Groups 2a and 2b (elderly subjects)

Forty-eight hours after the injection of benzathine bipenicillin, the long-acting penicillin complex, the mean concentrations were much higher in the elderly subjects (group 2b) than those observed after the injection of benethamine penicillin (table II). However, while the curve of mean concentrations for the latter preparation fell slowly, indicating a longer duration, the curve for benzathine bipenicillin fell

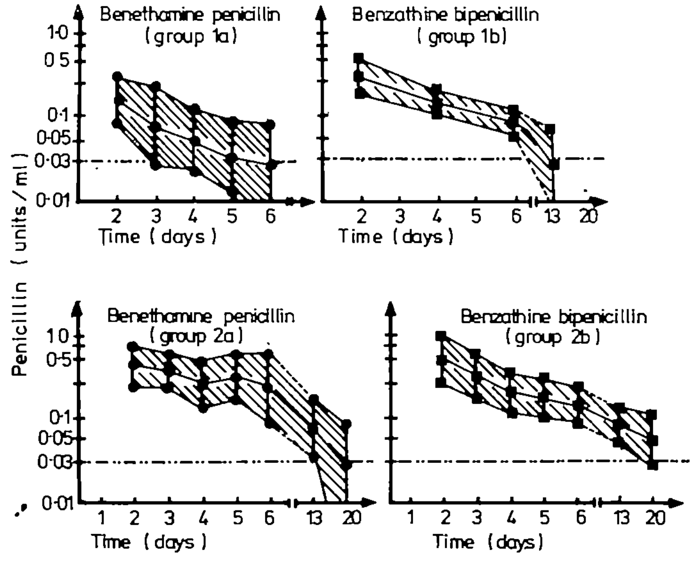


FIG 3 *Graphs of the mean serum concentrations of benethamine penicillin and benzathine bipenicillin in four groups of subjects (groups 1a and 1b, young subjects; groups 2a and 2b, elderly subjects).*

much more rapidly and crossed the other curve after about three days, so that the serum concentrations were lower than those obtained with the medium-long-acting penicillin (benethamine) (fig 3).

As before, the mean serum concentration and the calculated value of Ƶ are presented in table III and fig 1. Analysis of these results showed that after a single injection of 2·4 million IU of benzathine bipenicillin or 3 million IU of benethamine penicillin, the penicillin concentrations were very similar after 48 hours (no statistically significant difference) but subsequently became significantly higher for benethamine penicillin.

Discussion

Many factors are known to affect the action of drugs in the body; the processes of absorption, distribution, localisation in tissues, biotransformations, and excretion[43-45] consequently make a difference in their bioavailability. Some of these factors depend on physicochemical properties of the formulations; others are individual factors, such as weight, size, body surface, genetic factors, race, sex, and age.[18,25,28,46] We therefore considered that it would be interesting in this study to take into account only the age factor, which covers all these variations. Age modifies the kinetics of antibiotics. In children, as in elderly subjects, the serum concentrations of these products are higher than those observed in adults.[47-50]

Investigations on penicillin carried out with short-acting penicillins (those eliminated within a few hours) prescribed in a single dose, whatever the route of administration, corroborated our results.

Molholm-Hansen *et al*[19] demonstrated that after the injection of 600 mg (1 million IU) by the intravenous route the half-life of penicillin G in old people was twice the figure observed in young adults. Simon *et al*[20] confirmed these results in a study of 1 million units of propicillin given orally to young and old people. The same difference was observed by Leikola and Vartia[21] with penicillin G and procaine penicillin, by Clini *et al*[22] with ampicillin, and by Bouchon[23] after an intramuscular injection. Thus, penicillins with short elimination (<24 hours), as well as long-acting penicillins, are found at higher concentrations in the serum of elderly subjects than in that of young people.

It is an important factor that with increasing age all the functional capacities (respiratory, cardiac, and renal) are progressively being reduced. There is a more or less linear decrease of organic functions in old people.[48] Tissues are modified both in quality and quantity; the adipose tissue is increased and the body water (total, extracellular and intracellular) is reduced. As metabolically active tissues are progressively diminishing, the physical and biochemical compositions of quantitatively important tissues (such as the connective tissue) are modified[51]; the apparent volume of distribution is changed. The fixation on proteins[52-54] and on red blood cells[55] is modified. The metabolism and the elimination of drugs are different.[44,51]

On the other hand, in this study, when serum concentrations produced by 2·4 million IU of benzathine penicillin complex are compared with those produced by 3 million IU of benethamine penicillin complex, it appeared that with the latter (a) the sublethal concentrations were reached more quickly in young subjects; (b) penicillin concentrations <0·03 units/ml occurred sooner and more frequently (table II, figs 2 and 3); (c) the results of the penicillinaemia obtained were more dispersed about the mean (table II); (d) in weighted values the mean deviations in young subjects were homogeneous and comparable with those obtained with the benzathine penicillin complex (table III) while in elderly subjects variations were observed (table II) for both complexes; (e) the kinetics of elimination of both complexes were comparable in young subjects while in elderly subjects the benethamine penicillin complex followed a slower kinetic of elimination giving higher penicillin concentrations in serum for a longer period than the benzathine penicillin complex; and (f) treatment regimens based solely on the number of international units of penicillin were precarious if the properties of the chosen salts of penicillin were not taken into account.

According to work carried out on Gram-positive organisms and reported by Tinelli,[56] penicillin acts on growing organisms on the cell wall; the transpeptidase, involving the formation of interpeptidic bridges which fasten the reticulated structure of the cell wall, is inhibited. The growing bacteria then begin to swell and form protoplasts, which lyse as a result of osmotic pressure. Although *T pallidum* is not cultivatable in vitro the mechanism of the action of penicillin is considered to be the same (Luger A, paper read at the 14th International Congress of Dermatology, Venice, 1972; Kern A, personal communication, 1973).[57]

Based on this hypothesis, principles of standard penicillin treatment of early syphilis were established on the following syllogism: (a) all treponemes would divide every 30-33 hours[42]; (b) penicillin acts on these organisms when they divide (Kern A, a personal communication, 1973)[2,57] and when this antibiotic has risen to a concentration of 0·03 units/ml in serum[39,41,42]; and (c) therefore such a serum concentration maintained for 7-10 days is considered adequate to kill all the *T pallidum* which have invaded an organism.[2]

The results of our study have shown that for young subjects, with the standard dosages generally used in the treatment of syphilis when one or other of these complexes is used, it would be preferable to use benzathine bipenicillin, assuming the minimum active penicillin concentration to be 0·03 units/ml. But if we refer to the work of Eagle *et al*[58] the mean active concentration of penicillin in rabbits would be between 0·0083 units/ml and 0·0167 units/ml, but the effective concentration would be much higher—that is, 0·6 units/ml, an amount which is rarely attained with the two standard posologies used.

If it is admitted that a serum concentration of 0·03 units of penicillin per ml should be maintained for 7-10 days, it appeared that the benzathine bipenicillin complex did not give an adequate safety margin covering possible individual variations and the lack of direct relationship between serum concentrations and the therapeutic effect.[59-61]

Since most of the *T pallidum* are disseminated very quickly in the organism,[62] it seems unlikely that they would divide all together at the same time[37 57] and continuously at the same rhythm during the first days of the experimental infection,[62] since the multiplication time (30-33 hours) has been estimated at the local area of inoculation during the incubation period and in optimum conditions.[42]

This study corroborates the principle that the therapeutic activity is conditioned by the ability to keep an adequate concentration of the active part of the drug at the focus of infection for an adequate period of time; however, it must be noted that these therapeutic dosages given in a single injection do not allow any safety margin,[1 2] particularly as we do not have an absolute criterion which allows us to assert that all *T pallidum* have really been destroyed.[37]

It therefore appears from our results that it is not possible to achieve 100% bacteriological sterilisation in young subjects; dosages of this kind might be sufficient to heal a chancre but not to cure the host, which has been invaded by treponemes for a certain time, as we have proved[63 64] and as has been confirmed by others.[65 66]

Consequently, in the treatment of syphilis, a standard dose consisting of a single injection of a penicillin product (medium-long-acting or long-acting) should lead to a substantial number of therapeutic failures which seem to be due to the low dosages injected, which allow no safety margin.

In this respect, the selection of a treatment schedule should depend on the practical circumstances of the patient, as well as on the medical considerations, provided the dose/time principle is known and followed.[2]

We wish to thank Professor M Bolgert and Dr Garrel for their invaluable help, and S Burtchaell for his kind co-operation. Dr J Durel carried out the statistical analysis of the results.

References

1. Willcox, RR. Treatment of early venereal syphilis. *Br J Vener Dis* 1962;**38**:109-23.
2. Idsøe O, Guthe T, Willcox RR. Penicillin in the treatment of syphilis. *Bull WHO* 1972;**47** suppl:5-68.
3. Neuman M. *Vade-mecum des Antibiotiques et Agents Chimiothérapiques Anti-infectieux.* 1st and 4th ed. Paris: Maloine, 1962, 1979.
4. Thompson SE, Wiesner PJ, Sanford JP. Why review syphilis therapy now? *J Amer Ven Dis Assoc* 1972;**3**, No 2 (part 2):48.
5. Nelson MG, Talbol JM, Binns TB. Benethamine penicillin: a new salt with a prolonged action. *Br Med J* 1954;ii:339-41.
6. Bash B, Ireland DM, Thorn AG, Tomich EG. The pharmacological properties of benethamine penicillin. *Antibiot Chemother* 1955;**5**:152-61.
7. Huriez C, Desmons F, Fossati-Delescluse, R. Etude thérapeutique et métabolique de la bénéthamine-pénicilline. *Bull Soc Fr Dermatol Syphilol* 1964;**71**:643-4.
8. Szabo JL, Edwards CD, Bruce WF. N-N'dibenzylethylenediamine penicillin G: preparation and properties. *Antibiot Chemother* 1951;**1**:499-503.
9. Rein CR, Buckwalter FH, Mann C, Landy SE, Flax S. Time-dosage relationship in the treatment of treponemal diseases with a new combination of three penicillin salts. Laboratory and clinical basis for effective therapy. *J Invest Dermatol* 1953;**21**:435-46.
10. Tinkler AE, Shannon R. Daily penicillin serum concentrations following injection of 2·4 megaunits of "all-purpose" penicillin. *Bull WHO* 1966;**35**:857-62.
11. Graciansky P de, Grupper C. Résultats du traitement de la syphilis primo-secondaire par l'injection unique de pénicilline long-retard (à propos de 147 malades). *Bull Soc Dermatol Syphilol* 1959;**5**:699-710.
12. Graciansky P de, Grupper C. La bénéthamine dans le traitement des syphilis récentes. *Bull Soc Dermatol Syphilol* 1963;**70**:17.
13. Brown WJ. Syphilis and gonorrhea in general practice. *Clin Med Winnetka* 1962;**69**:91-100.
14. Siboulet A, Busquet PY, Egger L, Majewski E. Gonococcie et syphilis. *Bull Soc Dermatol Syphilol* 1973;**80**:642-5.
15. United States Public Health Service. *Syphilis: Modern Diagnosis and Management.* Washington DC: US Department of Health, Education and Welfare, 1960.
16. United States Public Health Service. *Syphilis: A Synopsis.* Public Health Service Publication No 1660. US Department of Health, Education and Welfare 1968:110-6.
17. United States Public Health Service. Center for Disease Control recommended treatment schedules for syphilis. *Curr Lit Vener Dis* 1976;**1**:8-10.
18. Dayer P, Balant L. La pharmacologie clinique pourquoi? *Med Hyg* 1978;**36**:997-8.
19. Molholm-Hansen J, Kampmann J, Laursen H. Renal excretion of drugs in the elderly. *Lancet* 1970;i:1170.
20. Simon C, Malerczykiv V, Muller U, Muller G. Zur pharmakokinetik von propicillin bei geriatrischen patienten in vergleich zujungeren eerwaschsenen.' *Dtsch Med Wochenschr* 1972;**52**:1999-2003.
21. Leikola E, Vartia KO. On penicillin levels in young and geriatric subjects. *J Gerontol* 1957;**12**:48-52.
22. Clini V, Mars G, Tansini G. La distribuzione di alcuni antibiotici in soggeti di varia eta. *La Settimana Medica* 1964;**52**:1007-24.
23. Bouchon JP. L'emploi des antibiotiques chez les vieillards. In: Bourlière F, ed. *Progrès en Gerontologie.* Paris: Editions Médicales Flammarion 1969:249-263.
24. Food and Drug Administration. Proposed bioavailability requirements. *Federal Register* 1973;**38**:885-7.
25. Chodos DJ, Disanto AR. Basics of bioavailability and description of Upjohn single dose study design. *Upjohn Company Ed.* Kalamazoo, Michigan; Upjohn Co, 1974.

26. Hirtz J. La disponibilité biologique des médicaments. *Actual Pharmacol* 1976;**28**:76-106.
27. Dagher *et al.* Nomogramme pour la détermination des volumes sanguin, plasmatique et globulaire à partir de l'âge et du poids corporel de l'adulte. *Adv Surg* 1965;**1**:69.
28. Hirtz J, Schoeller JP. Intérêt clinique du dosage des médicaments dans le sang. *Nouv Presse Med* 1976;**5**:869-72.
29. Singlas E. Biodisponibilité. In: Giroud JP, Mathé G, Meyneil G, eds. *Pharmacologie Clinique.* Paris: Expansion Scientifique 1978:119-35.
30. Schumacher GE. Practical pharmacokinetic techniques for drug consultation and evaluation: Use of dosage regimen calculations. *Am J Hosp Pharm* 1972;**29**:474-84.
31. Chabbert Y, Boulingre H. Modifications pratiques concernant le dosage des antibiotiques en clinique. *Rev Fr Etudes Clin Biol* 1957;**1**:636-40.
32. Lightbown JW. Svlitzeanu D. The assay of penicillin in blood-serum using *Sarcina lutea. Bull WHO* 1957;**17**:553-67.
33. Bennet JV, Brodie JL, Benner EJ, William MMK. Simplified accurate method for antibiotic assay of clinical specimens. *Appl Microbiol* 1966;**14**:170-7.
34. Bliss CI. In: *Statistics in Biology*. New York: McGraw Hill Inc, 1970.
35. Brogard JM, Pinget M, Dorner M, Lavillaureix J. Etablissement du profil pharmacocinétique d'un antibiotique. Intérêt pratique et applications à l'étude comparative des céphalosporines. *Bull Inst Pasteur* 1976;**74**:383-412.
36. Conover WJ. In: *Practical Non-parametric Statistics.* New York: John Wiley and Son Inc, 1976.
37. Collart P. Critique des éléments de base sur lesquels reposent certains traitements de la syphilis. *Sem Hop* 1974;**50**:673-9.
38. Eagle H, Magnuson HJ, Fleischman R. Relation of the size of the inoculum and the age of the infection to the curative dose of penicillin in experimental syphilis with particular reference to the feasibility of its prophylactic use. *J Exper Med* 1947;**85**:423-40.
39. Eagle H. Speculations as to the therapeutic significance of the penicillin blood level. *Ann Intern Med* 1948;**28**:260-78.
40. Eagle H. The dynamics of penicillin action to its use in the treatment of syphilis. Recapitulation of the experimental data. In: *Symposium on Current Progress in the Study of Venereal Diseases.* Washington DC: Division of Venereal Diseases, United States Public Health Service, 1949:272-6.
41. Magnuson HJ. The use of antibiotic in spirochaetosis laboratory and general considerations. *Am NY Acad Sci* 1952;**55**:1161-7.
42. Turner TB, Hollander MD. Biologie des tréponématoses. *Monograph Series* No 35. Geneva: WHO, 1957.
43. Eben-Moussi E, Van den Driessche J. Pharmaco-cinétique des antibiotiques dans l'organisme humain. *Anest Anal Réan* 1971;**28**:671-87.
44. Boissier JR, Advenier C. Résorption des médicaments. *Concours Médical* 1976;**98**:6613-73.
45. Benazet F, Dubost M, Jolles G, Leau O, Pascal C, Preud'Homme J, Terlain B. Liaisons et modifications des antibiotiques in vivo (sang et foyer infectieux). *Biol Med* 1968;**57**:1-26.
46. World Health Organisation. Biodisponibilité des médicaments: Principes et problèmes. *WHO Tech Rep Ser* 1974;No 536.
47. Gladtke E. Pharmacokinetics in relation to age. *Boll Chim Farm* 1973;**112**:333-41.
48. Vignalou J. Généralités sur la thérapeutique en gériatrie. *Cah Med* 1974;**15** (9), 573-8.
49. Bender AD, Pharmacodynamic principles of drug therapy in the aged. *J Am Geriatr Soc* 1974;**22**:296-303.
50. Gorrod JW. Absorption, metabolism and excretion of drugs in geriatric subjects. *Clin Gerontol* 1974;**16**:30-42.
51. Paychere JM. Géronto-pharmacologie. *Médecine et Hygiène* 1978;**36**:1043-53.
52. Kunin CM. Clinical pharmacology of the new penicillin. I: The importance of serum protein binding in determining antimicrobial activity and concentration in serum. *Clin Pharmacol Ther* 1966;**7**:166-79.
53. Rolinson GN. The significance of protein binding of antibiotics in vitro and in vivo. In: Waterson AP, ed. *Recent Advances in Medical Microbiology,* Chap 7. London: J & A Churchill, 1967:254-83.
54. Wallace S, Whiting B, Muncio J. Factors affecting drug binding in plasma of elderly patients. *Br J Clin Pharmacol* 1976;**3**:327-30.
55. Chan K, Kendall MJ, Ritchard M, Welles WDE, Wickers MD. The effect of aging on plasma pethidine concentration. *Br J Clin Pharmacol* 1975;**2**:197-302.
56. Tinelli R. Le glycopeptide des parois bactériennes. *Bull Inst Pasteur* 1968;**66**:2507-38.
57. Luger A. Diagnostik und therapie der syphilis. *Med Klin* 1971;**66**:339-43.
58. Eagle H, Fleischman R, Musselman AD. The effective concentration of penicillin in vitro and in vivo for streptococci, pneumococci and *Treponema pallidum. J Bacteriol* 1950;**59**:625-43.
59. Heite HJ. Beitrag zur verteilung des penicillins im savgetierorganismus. *Klin Wochenschr* 1952;**30**:174-5.
60. Anonymous. Oral penicillins, blood levels, antimicrobial sensitivity and clinical effectiveness. *JAMA* 1961;**175**:607-8.
61. Balant L, Dayer P. Il n'y a pas de relation entre les taux sanguins et l'effet du médicament. *Médecine et Hygiene* 1978;**36**:1029-38.
62. Collart P. Le tréponème pâle. *Rev Méd* 1970;**11**:1265-300.
63. Collart P, Poitevin M. Le problème des tréponèmes pénicillinorésistants. *Sem Hop Paris* 1975;**51**:1489-96.
64. Collart P, Poitevin M. Données récentes sur le comportement des tréponèmes pâles. *Revue du Praticien (Paris)* 1976;**26**:4065-76.
65. Giles AJM, Lawrence AG. Treatment failure with penicillin in early syphilis. *Br J Vener Dis* 1979;**55**:62-4.
66. Lanigan-O'Keeffe FM. Monorecidive or chancre redux. *Br J Clin Pract* 1978;**82**:206-7.

Br J Vener Dis 1980;**56**:363-7

Penicillin concentrations in cerebrospinal fluid after different treatment regimens for syphilis

NIWAT POLNIKORN,* RAWIPHAN WITOONPANICH,* MALAI VORACHIT,† SODSAI VEJJAJIVA,‡ AND ATHASIT VEJJAJIVA*

*From the Departments of *Medicine and †Pathology, Faculty of Medicine, Ramathibodi Hospital, and the ‡Department of Microbiology, Faculty of Medicine, Chulalongkorn Hospital, Bangkok, Thailand*

SUMMARY The concentrations of penicillin in the cerebrospinal fluid (CSF) were compared simultaneously with those in the serum in 17 patients with syphilis. The antibiotic concentrations were measured by the agar well diffusion method. There were no detectable concentrations of penicillin in the CSF after administration of benzathine penicillin 2·4 megaunits, benzathine penicillin 7·2 megaunits, procaine penicillin in aluminium monostearate (PAM) 12 megaunits, or aqueous procaine penicillin G 2·4 megaunits. Only after high doses of aqueous penicillin G 24 megaunits daily or aqueous penicillin G 2 megaunits daily together with oral probenecid 2 g daily was penicillin detectable in the CSF. The concentrations after the latter regimen were the highest and much higher than the minimum inhibitory concentration for *Treponema pallidum.*

Introduction

Even though penicillin is the drug of choice in the treatment of syphilis,[1] the results in the late stages, especially in neurosyphilis, are still not perfect.[2 3] Viable treponemes have been detected after treatment with the recommended dosage of penicillin.[4-7]

Mohr *et al*[8] reported that 12 of their 13 patients who received benzathine penicillin G intramuscularly (im) for the treatment of neurosyphilis had no detectable penicillin in the cerebrospinal fluid (CSF) and suggested that the drug might have to be given intravenously (iv) to produce an effective concentration in the CSF. Yoder[9] reported that in one patient treated with procaine penicillin G 600 000 IU by daily intramuscular injection the drug concentration in the CSF after five days was less than 0·017 IU/ml. The minimum fully treponemicidal concentration of penicillin recommended by the World Health Organisation is 0·03 IU (0·018 μg) per ml.[10] For effective treatment of neurosyphilis it seems logical that this concentration should be achieved in the CSF. Boger *et al*[11] found that by giving penicillin intramuscularly together with oral carinamide, which inhibits renal tubular excretion of penicillin, the concentrations in the CSF of patients with neurosyphilis were higher than when penicillin was given alone.

The purpose of this study was to measure the penicillin concentrations in the CSF of patients with syphilis after different recommended dosages of penicillin and to compare them with those after a new regimen using parenteral penicillin together with oral probenecid.

Patients and methods

From January 1978 all patients who were diagnosed as having secondary, latent, and late syphilis and neurosyphilis at Ramathibodi Hospital were studied. The diagnosis was based on history and clinical examination together with serological and CSF findings. All patients were informed of the study and the necessity for repeated lumbar punctures. Signed consent was obtained.

SEROLOGICAL TESTS

The Venereal Disease Research Laboratory (VDRL) test was performed by the standard method,[12] the fluorescent treponemal antibody absorption (FTA-ABS) test by the method of Hunter *et al,*[13] and the *Treponema pallidum* haemagglutination assay (TPHA) by the method of Rathlev.[14] The CSF-FTA, CSF-FTA-ABS, and CSF-FTA-IgM tests were performed on undiluted specimens.

Address for reprints: Dr Niwat Polnikorn, Division of Dermatology and Venereology, Ramathibodi Hospital, Rama VI Road, Bangkok, Thailand

Received for publication 7 January 1980

TABLE I *Penicillin concentrations in serum and CSF of 17 patients with syphilis*

	Treatment regimen		*Penicillin concentration*		
Case No	*Drug*	*Dose*	*Time measured (days after last dose)*	*Serum (IU/ml)*	*CSF (IU/ml)*
1	Benzathine penicillin	2·4 mu (im)	3rd	0·043	0
2	Benzathine penicillin	2·4 mu (im)	3rd	0·007	0
3	Benzathine penicillin	7·2 mu (im) (2·4 mu × 3 doses)	3rd	0·016	0
4	Benzathine penicillin	7·2 mu (im) (2·4 mu × 3 doses)	3rd	0·45	0
5	Benzathine penicillin	7·2 mu (im) (2·4 mu × 3 doses)	3rd	0·45	0
6	Benzathine penicillin	7·2 mu (im) (2·4 mu × 3 doses)	3rd	0·255	0
7	PAM and Benzathine penicillin	4·8 mu (im) 4·8 mu (im)	3rd	0·023	0
8	PAM	12 mu (im) (1·2 mu × 10 doses)	30 min	0·991	0
9	Aqueous penicillin G	400 000 IU (iv) (4 hrly × 10 days)	30 min (after 1st dose) 15 min (before last dose)	1·015 0·962	0 0
10	Aqueous penicillin G	400 000 IU (iv) (4 hrly × 7 days)	30 min (after last dose) 15 min (before last dose)	2·979 0·160	0 0
11	Aqueous penicillin G	4 mu (iv) (4 hrly × 10 days)	30 min (after 1st dose) 15 min (before next dose) 15 min (before last dose)	56·05 128·25 3·453	0·044 0·355 0·579
12	Aqueous penicillin G+ Probenecid	500 000 IU (iv) 500 mg (orally) (6 hrly × 20 days)	5th (of treatment)* 10th (of treatment)* 20th (of treatment)*	6·05 6·08 8·238	2·838 1·611 1·84
13	Aqueous penicillin G+ Probenecid	500 000 IU (iv) 500 mg (orally) (6 hrly × 20 days)	5th (of treatment)* 10th (of treatment)* 20th (of treatment)*	3·565 4·835 5·787	0·414 0·191 0·231
14	Aqueous penicillin G+ Probenecid	500 000 IU (iv) 500 mg (orally) (6 hrly × 20 days)	5th (of treatment)* 10th (of treatment)*	3·7 4·0	0·45 0·5
15	Aqueous penicillin G+ Probenecid	500 000 IU (iv) 500 mg (orally) (6 hrly × 20 days)	5th (of treatment)* 10th (of treatment)* 20th (of treatment)*	4·35 4·9 1·54	0·447 0·3 0·12
16	Aqueous penicillin G+ Probenecid	500 000 IU (iv) 500 mg (orally) (6 hrly × 20 days)	5th (of treatment)* 10th (of treatment)* 20th (of treatment)*	3·48 4·8 2·1	0·35 0 0
17	Aqueous penicillin G+ Probenecid	500 000 IU (iv) 500 mg (orally) (6 hrly × 20 days)	5th (of treatment)* 20th (of treatment)*	1·85 2·5	0 0·85

*Three hours after first dose
mu = megaunits
PAM = procaine penicillin in aluminium monostearate

SAMPLE COLLECTION

Serum and CSF samples were collected simultaneously before, during, and after treatment according to the regimen shown in table I. In patients who received benzathine penicillin G the samples were collected on the third day after injection whereas in those who received high-dose aqueous penicillin G they were collected 30 minutes after the first dose and 15 minutes before the last dose. In patients treated with aqueous penicillin G and oral probenecid the specimens were collected on the fifth, tenth, and twentieth days of treatment three hours after the first dose of that day.

PENICILLIN CONCENTRATIONS

The penicillin concentrations in the serum and CSF were measured by microbiological assay using an agar well diffusion method with *Bacillus subtilis* ATCC 6633 as the test organism.[15] The accuracy of this method is more than 95% confidence limit. The minimum concentration of penicillin that can be detected by this method is 0·006 IU/ml. The serum and CSF specimens were stored at −20°C if the assay could not be carried out immediately.

CLINICAL PRESENTATION

The clinical presentation of the 17 patients studied is summarised in table II. Seven patients had early syphilis (cases 1, 3, 5, 6, 7, 13, and 17), three occlusive vascular diseases (cases 2, 11, and 12), one epilepsy (case 16), one general paralysis of the insane (case 15), and one tabes dorsalis (case 10), while three were asymptomatic (cases 4, 9, and 14) and one had congenital syphilis with chronic urticaria (case 8).

TREATMENT

The treatment schedules are given in table I. Case 7 was given a combination of procaine penicillin and aluminium monostearate (PAM) and benzathine penicillin G (total 9·6 megaunits im) because of panniculitis after the second dose of PAM. The combination of aqueous penicillin G 500 000 IU iv together with probenecid 500 mg orally given 30 minutes before injection every six hours for 20 days was studied in cases 12-17.

Results

The penicillin concentrations in serum and CSF of the 17 patients studied are shown in table I. None had detectable penicillin concentrations in the control specimens. In those patients who received benzathine penicillin G the serum concentrations on the third day after the last dose ranged from 0·0067 to 0·45 IU/ml whereas there were no detectable concentrations in the CSF. With PAM the serum concentration after 12 megaunits was higher (0·9912 IU/ml) but still no penicillin was detected in the CSF. Even with aqueous penicillin G 400 000 IU iv, which gave sufficiently high serum concentrations

TABLE II *Clinical presentation, serological test results, and CSF findings in 17 patients with syphilis*

				Serum			*CSF*				
Case No	*Sex*	*Age (years)*	*Clinical presentation*	*VDRL test (titre)*	*TPHA test*	*FTA-ABS test*	*WC* (/mm³)*	*Protein (g/l)*	*FTA test*	*FTA-ABS test*	*FTA-IgM test*
1	M	25	Skin rash	1/32	R	R	1	0·68	R	NR	NR
2	F	47	Cranial nerve III palsies	1/1	R	ND	0	0·25	NR	NR	NR
3	M	22	Skin rash	1/16	ND	R	0	0·48	R	NR	NR
4	M	22	Asymptomatic	1/2	R	R	1	0·38	R	NR	NR
5	M	25	Alopecia	1/16	R	R	1	0·54	R	NR	NR
6	M	25	Skin rash	1/64	ND	R	0	0·45	NR	NR	NR
7	F	25	Alopecia	1/64	R	R	1	0·52	R	NR	NR
8	F	29	Chronic urticaria	1/2	ND	R	5	0·56	NR	NR	NR
9	M	28	Asymptomatic	1/4	R	R	5	0·52	R	NR	NR
10	M	48	Tabes dorsalis	1/4	ND	ND	46	0·40	R	R	NR
11	F	68	Cranial nerves III and IV palsies	1/8	R	R	12	0·22	R	NR	NR
12	F	70	Brain stem vascular disease	1/16	R	R	2	0·49	NR	NR	NR
13	M	24	Alopecia, absent deep tendon reflexes	1/64	R	R	0	0·36	R	R	NR
14	M	50	Asymptomatic	1/32	R	R	0	0·54	R	R	R
15	M	54	General paralysis of the insane	1/2	R	R	4	0·58	R	R	NR
16	M	46	Epilepsy	1/1	R	R	†	†	R	NR	NR
17	F	38	Skin rash	1/128	R	R	0	0·52	R	R	R

*WC = White cells (lymphocytes)
†Data not recorded
R = reactive, NR = non-reactive, ND = not done

(1·015-2·979 IU/ml at 30 minutes after the first dose), no penicillin was detectable in the CSF.

Only with a high dose of aqueous penicillin—that is, 4 megaunits iv every four hours, giving a serum concentration of 56·05 IU/ml at 30 minutes after the first dose—was the drug detected in the CSF (0·044 IU/ml). With this regimen—even at 15 minutes before the last dose, which theoretically was the time of low serum concentration (3·453 IU/ml)—a high penicillin concentration could still be detected in the CSF (0·579 IU/ml).

Interestingly, patients receiving aqueous penicillin G 500 000 IU iv with oral probenecid 500 mg 30 minutes before injection every six hours showed high serum concentrations ranging from 1·85 to 6·05 IU/ml (mean 3·833 IU/ml) with simultaneous CSF concentrations ranging from 0 to 2·838 IU/ml (mean 0·75 IU/ml) at three hours after the first dose of penicillin on the fifth day of treatment. The mean CSF concentrations of penicillin at three hours after the first dose on the tenth and twentieth days were 0·521 and 0·608 IU/ml respectively whereas the simultaneous serum concentrations were 4·923 and 4·033 IU/ml respectively.

The CSF concentrations as the mean percentage of the serum concentrations were 19·57% on the fifth day, 10·58% on the tenth day, and 15·08% on the twentieth day.

Discussion

Although penicillin is still the drug of choice for the treatment of all stages of syphilis, many reports[16-20] of failure to eradicate the organism or to halt the progression of the disease in later stages—in particular, neurosyphilis—have been of recent interest. In some reports methodological difficulties raised uncertainties,[21] but there were at least six which documented success in producing darkfield-positive lesions containing typical treponemes in animals after the inoculation of materials from penicillin-treated patients with syphilis.[4 6 7 22-24]

Our findings confirm those of Boger *et al*,[11] who found that with aqueous penicillin G 100 000 IU im three-hourly low concentrations of penicillin (ranging from 0·019 to 0·052 IU/ml) could be detected in the CSF by the Rammelkamp serial dilution method, but after the addition of oral carinamide 3 g three-hourly the detectable concentrations of penicillin increased to between 0·026 and 2·5 IU/ml. Carinamide, like probenecid, delays the excretion of penicillin by inhibiting renal tubular function.

In our patients there was no detectable penicillin in the CSF after recommended doses of benzathine penicillin G and PAM or even after aqueous penicillin G 2·4 megaunits iv daily. After high doses of aqueous penicillin G (24 megaunits iv daily) sufficient penicillin could be detected in the CSF but the concentrations were lower than those obtained after aqueous penicillin G (2 megaunits iv daily) together with oral probenecid (2 g daily).

Apart from the benefit of lower doses of penicillin in the new regimen it seems likely that probenecid may have a direct effect on the blood-brain barrier, thus enhancing penetration of penicillin into the central nervous system. This however needs further evaluation.

Recently, Dunlop *et al*,[25] studying 31 patients and using single lumbar punctures to determine the concentration of penicillin in the CSF on different days after treatment but with no control specimens for the penicillin concentration, obtained similar results to ours. Only after aqueous penicillin G 500 000 IU im six hourly together with probenecid 500 mg six hourly by mouth in six of eight patients were treponemicidal concentrations of penicillin in the CSF achieved.

It is logical that treponemicidal concentrations of penicillin in the CSF should be obtained in the treatment of neurosyphilis. However the outcome of treatment with this new regimen compared with those previously used needs a larger number of patients to be studied and a longer period of follow-up before definite benefits can be established.

References

1. Center for Disease Control. Recommended treatment schedules 1976. *Morbidity and Mortality Weekly Report* 1976;**25**:105-7.
2. Smith CA, Kamp M, Olansky S, *et al.* Cited by King AJ. *Recent Advances in Venereology WHO/VDT/141* London: J & A Churchill Ltd, 1964:105.
3. Short DH, Knox JM, Clicksman J. Neurosyphilis: the search for adequate treatment. *Arch Dermatol* 1966;**93**:87-91.
4. Collart P, Borel L, Durel P. Etude de l'action de la penicilline dans la syphilis tardive; Persistence du tréponème pale aprés traitment. *Ann Inst Pasteur* 1962;**102**:596-615.
5. Dunlop EM. Persistence of treponemes after treatment. *Br Med J* 1972;ii:577-80.
6. Hardy JB, Hardy PH, Oppenheimer EH, Ryan SJ, Shelf RN. Failure of penicillin in a newborn with congenital syphilis. *JAMA* 1970;**212**:1345-9.
7. Tramont EC. Persistence of *Treponema pallidum* following penicillin G therapy: report of two cases. *JAMA* 1976;**236**:2206-7.
8. Mohr JA, Griffiths W, Jackson R, *et al.* Neurosyphilis and penicillin levels in cerebrospinal fluid. *JAMA* 1976;**236**:2208-9.
9. Yoder FW. Penicillin treatment of neurosyphilis. *JAMA* 1972;**232**:270-1.
10. Idsøe O, Guthe T, Willcox RR. Penicillin in the treatment of syphilis. *Bull WHO* 1972;**47**:suppl 9:6-13.
11. Boger WP, Baker RB, Wilson WW. Penicillin in the cerebrospinal fluid following parenteral penicillin. *Proc Soc Exp Biol Med* 1948;**68**:101-6.
12. United States Public Health Service. *Manual of Serologic Tests for Syphilis.* Publication No 411. Washington DC: US Department of Health Education and Welfare, 1969.
13. Hunter EF, Deacon WE, Meyer PC. *Publ Hlth Rep* 1964;**79**:410-2.

14. Rathlev T. Haemagglutination test utilizing pathogenic *Treponema pallidum* for the serodiagnosis of syphilis. *Br J Vener Dis* 1967;**43:**181-5.
15. Bennett JV, Brodie JL, Benner EJ, Kirby WM. Simplified accurate method for antibiotic assay of clinical specimens. *Appl Microbiol* 1966;**14:**170-7.
16. Smith JL. In: *Spirochetes in Late Seronegative Syphilis, Penicillin Notwithstanding*. Springfield, Illinois: Charles C Thomas, 1969.
17. Kaplan JM, McCracken H. Clinical pharmacology of benzathine penicillin G in neonates with regard to its recommended use in congenital syphilis. *J Pediatr* 1973;**82:**1069-72.
18. Nichols FT, Heyman A. Penicillin therapy in asymptomatic neurosyphilis: A comparision of amorphous penicillin, penicillin in oil and wax, and crystalline penicillin G. *American Journal of Syphilis, Gonorrhoea and Venereal Diseases* 1949;**33:**561-70.
19. Dattner B. Penicillin failures in neurosyphilis. *American Journal of Syphilis, Gonorrhoea and Venereal Diseases* 1949;**33:**571.
20. Wilner E, Brody JA. Prognosis of general paresis after treatment. *Lancet* 1968;**ii:**197-205.
21. Montenegro ENR, Nicol WF, Smith JL. Treponema-like forms and artifacts. *Am J Ophthalmol* 1969;**68:**197-205.
22. Smith JL, Israel CW. Recovery of spirochetes in the monkey by passive transfer from human late seronegative syphilis. *Br J Vener Dis* 1968;**44:**109-15.
23. Yobs AR, Clark JR, Mothershed SE, *et al.* Further observation on the persistence of *Treponema pallidum* after treatment in rabbits and humans. *Br J Vener Dis* 1968;**44:**116-30.
24. Yogeswari L, Chacko CW. Persistence of *T pallidum* and its significance in penicillin-treated seropositive late syphilis. *Br J Vener Dis* 1971;**47:**339-47.
25. Dunlop EMC, Al-Egaily SS, Honang ET. Penicillin levels in blood and CSF achieved by treatment of syphilis. *JAMA* 1979;**241:**2538-40.

Br J Vener Dis 1980;**56**:368-71

Tabes dorsalis progressing to general paresis after 20 years despite routine penicillin therapy

A J H GILES

From the Department of Venereology, Pilgrim Hospital, Boston, Lincolnshire

SUMMARY A man with a history of treatment for early syphilis presented with tabes dorsalis. Despite receiving a course of penicillin accepted as adequate by the World Health Organisation the illness progressed to tabo-paresis after 20 years. Reinfection cannot be excluded.

Introduction

Authorities on the treatment of symptomatic neurosyphilis, although agreeing that penicillin is the drug of choice, vary considerably in their recommendation of both the total amount of penicillin to be administered and the duration of the treatment. Based on Hahn's[1] work, the World Health Organisation[2] maintains that 10-15 days' treatment with daily procaine penicillin is acceptable. This regimen is recommended by the United States Public Health Service,[3 4] by Morton and Harris,[5] and in standard textbooks on medicine.[6-10]

Glatt[11] recently questioned whether the amount of penicillin necessary to cure established neurosyphilis is yet known. King and Nicol,[12] Catterall,[13] and Brain[14 15] all recommended three weeks' treatment and Merritt,[16] 3-4 weeks.

Case report

FIRST ILLNESS

In 1957 a 52-year-old man was referred to the National Hospital for Nervous Diseases, Queen Square, London, at the suggestion of an optician whom he had attended for routine examination. He had no specific symptoms. He gave a past history of treatment for syphilis at St Mary's Hospital, London, in 1938 (records are no longer available).

Clinical findings

Findings on examination included a tabetic facies, bilateral optic atrophy, and Argyll-Robertson pupils. Higher mental functions were not impaired. There was generalised hypotonia, and the characteristic distribution of facial, sternal, and ulnar border hypoalgesia was noted. Deep pain sensation of the Achilles tendon was lost. Vibration sense was absent in both legs and joint-position sense was lost in the toes. Only the knee jerks were diminished; the Romberg test gave a negative result and the gait was normal. Signs of aortic regurgitation were noted—blood pressure of 150/60 mmHg, collapsing pulse, and a clearly audible to-and-fro aortic murmur.

Laboratory findings

The results of investigations before and after treatment are shown in table I. Penicillin 12 megaunits intramuscularly was given over a period of 10 days with no side effects. No further details were available, but procaine penicillin 1 megaunit daily was the accepted regimen at this hospital at that time.

Annual attendance at the outpatient department as well as postgraduate demonstrations and examinations are recorded until 1973. Apart from some deterioration of visual acuity no new symptoms developed and no further laboratory tests were performed.

SECOND ILLNESS

On 21 December 1977 this man, now aged 72 years, was admitted to the acute psychiatric ward at St Mary Abbots Hospital, London. He complained of unsteadiness but denied any other symptoms. The caretaker of his lodgings stated that over several weeks he had changed completely from being a quiet tenant to being totally distracted. At nights he became restless and agitated and complained that people were entering his room. He had taken to going out into the street at night in his pyjamas and shouting out.

Psychiatric history

On admission the patient complained of hearing voices at night which frightened him. He was

Address for reprints: Dr A J H Giles, Department of Venereology, Pilgrim Hospital, Sibsey Road, Boston, Lincs PE21 9QS

Received for publication 13 May 1980

TABLE I *Laboratory results during first illness*

	Blood test results			*CSF findings/test results*					
Date	*Kahn*	*Wassermann (units)*	*ESR (mm/hr)*	*Cells* (× 106/l)*	*Protein (g/l)*	*Wassermann (units)*	*Pandy*	*Nonne-Apelt*	*Lange*
17.11.57 At diagnosis	+	+ (28)	51	62	1·4	+ (15)	+	+	5555432111
21.7.58 8 months after treatment	+	−†		4	0·7	+ (4)	±	−	0011210000
23.5.59 18 months after treatment				6	0·7	−	+	±	0001211000

*Lymphocytes
†Not quite negative
+ Positive; ± weakly positive; − negative

disorientated and could not recall how he had arrived in the hospital bed. Long-term memory and proverb interpretation were impaired and he was unable to cope with simple arithmetical tasks.

This acute hallucinosis with disturbed behaviour was considered by the psychiatrist to be a very unusual presentation of tabo-paresis, which was suggested only when the various physical signs were noted. At this time his doctors were completely unaware of any past history; the patient only vaguely remembered being treated for syphilis in his youth.

Clinical findings

The physical findings were as before, with the addition of generalised muscle tremor, slurred speech, and a broad-based abnormal gait. His pupils were now unequal; the left was smaller than the right and there was a left-sided ptosis. Gross eighth nerve deafness was later detected by audiometry.

Progression and treatment

In the few days after admission the patient's condition deteriorated rapidly. He became unmanageable at night, was unable to dress or feed himself, and was doubly incontinent. A left-sided hemiparesis developed together with a pyrexia due to a urinary tract infection.

The laboratory findings at this time are shown in table II, together with those 15 months after a 10-day course of procaine penicillin. Within a week of starting treatment a remarkable clinical improvement occurred and the hemiparesis mysteriously disappeared. Unfortunately, the personality disorder did not completely resolve and two years later he remains a suspicious paranoid individual, although at times he can be a flamboyant, entertaining, and eccentric old gentleman.

Discussion

Cases of tabes progressing to general paralysis of the insane (GPI) were described in the pre-penicillin era, usually after insufficient treatment.[17,18] Cases of classical neurosyphilis after insufficient penicillin treatment for early syphilis have also been described recently.[19]

Cases of relapsing neurosyphilis after penicillin treatment have also been reported. Dattner[20] described instances of failure of the CSF to return to normal but maintained that once the findings in the CSF had become normal for a year relapses were almost unknown. The concept of Dattner and Thomas[21] in assessing the CSF is often quoted; this maintains that if the cell count becomes and remains within normal limits after treatment the disease is no longer active in the central nervous system. King and Nicol[12] commented that this should not be taken as "an absolute rule." Dowzenko and Krysztofiak[22] have reported two very unusual cases of relapsing neurosyphilis.

Wilner and Brody[23] reported that more than 50% of patients with GPI developed further neurological signs after penicillin treatment. This, however, is usually interpreted as scar-tissue injury rather than reactivation of the disease.

There have been reports that penicillin has failed to reach the CSF in spirochaeticidal concentrations during recommended treatment schedules with procaine penicillin in neurosyphilis[24,25] and that spirochaetal forms[26,27] and *Treponema pallidum* have persisted in the CSF after penicillin therapy.[28]

Despite these reports, present opinion remains confident that penicillin is still successful in preventing the development of further gross manifestations of neurosyphilis. This was what Martin affirmed in 1972.[29]

TABLE II *Laboratory results during second illness*

	Blood test results							*CSF findings/test results*					
Date	*TPHA (titre)*	*VDRL (titre)*	*FTA-ABS*	*RPCFT*	*FTA*		*ESR (mm/hr)*	*Cells* ($\times 10^6/l$)*	*Protein (g/l)*	*TPHA (titre)*	*VDRL (titre)*	*FTA-ABS (titre)*	*IgG† (IU/l)*
					IgG	*IgM*							
22.12.77 At diagnosis	+(1/5120)	+(1/32)	+	+	+	–	41	0‡	2·0	+	§	+	2·83
6.4.79 15 months after treatment	+(1/5120)	+(1/16)	+	+				4	0·7	+ (1/10 000)	+ (1/2)	+ (>1/40)	0·31

*Lymphocytes

†Normal value <0·05 IU/1.

‡Because of Christmas holiday CSF was probably not examined on the day of collection.

§Not accurately reported owing to contamination.

TPHA = *T pallidum* haemagglutination assay; VDRL = Venereal Disease Research Laboratory (test);
RPCFT = Reiter protein complement-fixation test; FTA-ABS = fluorescent treponemal antibody absorbed (test).

In conclusion, two authorities whose criteria for the assessment of cure in cases of neurosyphilis were first suggested 20 years ago stated: "The arrest of the disease can only be regarded as having been achieved when the CSF is normal and the VDRL is negative."[14 15] "In the absence of clinical progression and with tests of CSF which become and remain normal it is nevertheless a wise precaution to perform further tests of the CSF after a further two years."[12]

In the light of this case I think it reasonable to advise that if the CSF does not return to normal, lumbar puncture should be performed every two years and be repeated indefinitely; attendance at follow-up should be ensured.

I should like to thank Dr R Ross-Russell and Dr M de Mowbray, for their permission to report this case, and Mrs Mary Robinson for her work on the preparation of the manuscript.

References

1. Hahn RD. Some remarks on the management of neurosyphilis. *J Chronic Dis* 1961;**13**:1-5.
2. Idsøe O, Guthe T, Willcox RR. Penicillin in the treatment of syphilis. *Bull WHO* 1972;**47**, suppl 12:38-40.
3. Center for Disease Control. *VD Fact Sheet 1973*. Washington DC: United States Department of Health, Education and Welfare 74:8195. 1974:25.
4. Sparling PF. Diagnosis and treatment of syphilis. *N Engl J Med* 1971;**284**:650.
5. Morton RS, Harris JRW. *Recent Advances in Sexually Transmitted Diseases*. Edinburgh: Churchill Livingstone 1975:166.
6. Matthews WB, Miller H. *Diseases of the Nervous System*. 2nd ed. Oxford: Blackwell Scientific Publications 1975;196.
7. Holmes KK. Syphilis. In: Thorn GW, Adams RD, Braunwald E, Isselbacher KJ, Petersdorff RG, eds. *Harrison's Principles of Internal Medicine*. 8th ed. New York: McGraw-Hill Book Co, 1977;926.
8. Williams D. Infections of the meninges and the nervous system. In: Bodley Scott R, ed. *Price's Textbook of the Practice of Medicine*. 11th ed. London: Oxford University Press, 1973:1208.
9. Matthews WB. Infections of the nervous system. In: Bodley Scott R, ed. *Price's Textbook of the Practice of Medicine*. 12th ed. Oxford. Oxford University Press, 1978:1331.
10. Garrod LP, Lambert H, O'Grady F. *Antibiotic and Chemotherapy*. 4th ed. Edinburgh: Churchill Livingstone, 1973.
11. Glatt MM. Neurosyphilis yesterday and today. *Lancet* 1979;i:383.
12. King A, Nicol CS. *Venereal Diseases*. 3rd ed. London: Baillière Tindall, 1975:147, 154, 155.
13. Catterall RD. *A Short Textbook of Venereology*. 2nd ed. London: English Universities Press Ltd, 1974:159.
14. Brain WR, Walton JN. *Brain's Diseases of the Nervous System*. 7th ed. London: Oxford University Press, 1969:414.
15. Walton JN. *Brain's Diseases of the Nervous System*. 8th ed. Oxford. Oxford University Press, 1977:464.
16. Merritt HH. *A Textbook of Neurology*. 5th ed. Philadelphia: Lea & Febiger, 1973:135.
17. Stokes JH, Beerman H, Ingraham NR. *Modern Clinical Syphilology*. 3rd ed. Philadelphia & London: Saunders WB, 1945.
18. Kampmeier RH. *Essentials of Syphilology*. Oxford: Blackwell Scientific Publications, 1944:352.
19. Luxon L, Lees AJ, Greenwood RJ. Neurosyphilis today. *Lancet* 1978;i:90-3.
20. Dattner B. Penicillin failures in neurosyphilis. *Am J Syph Gonorr Vener Dis* 1949;**33**:571-5.
21. Dattner B, Thomas E. *The Management of Neurosyphilis*. New York: Grune & Stratton. London: Heinemann, 1944.
22. Dowzenko A, Krysztofiak B. Relapses in the CSF and acquired resistance to penicillin in cases of neurosyphilis. *J Neurol Sci* 1965;**2**:197-202.
23. Wilner E, Brody JA. Prognosis of general paresis after treatment. *Lancet* 1968;ii:1370-1.
24. Mohr JA, Griffiths W, Jackson R, *et al*. Neurosyphilis and penicillin levels in CSF. *JAMA* 1976;**236**:2208-9.
25. Yoder FW. Penicillin treatment of neurosyphilis. *JAMA* 1975;**232**:270-1.
26. Collart P, Borel L-J, Durel P. Study of the effect of penicillin in late syphilis: persistence of *Treponema pallidum* following treatment: 1 Late syphilis-experimental. *Ann Inst Pasteur* 1962;**102**:596-615.
27. Collart P, Borel L-J, Durel P. Significance of spiral organisms found after treatment in late human and experimental syphilis. *Br J Vener Dis* 1964;**40**:80-9.
28. Edmund C, Tramont MC. Persistence of *Treponema pallidum* following penicillin G therapy. *JAMA* 1976;**236**:2206-7.
29. Martin JP. Conquest of general paralysis. *Br Med J* 1972;i:159-60.

UNIVERSITY LIBRARY

Br J Vener Dis 1980;**56**:372-6

Immunological responses in late syphilis

R SHANNON,* C G COPLEY,* AND G D MORRISON†

*From the *Public Health Laboratory, Bristol, Avon; and the †Department of Sexually Transmitted Diseases, Royal Devon and Exeter Hospital (Wonford), Exeter, Devon*

SUMMARY Thirty-one serum samples from 18 patients with clinically established late syphilis and 1319 from patients at other stages of the disease were fractionated by density gradient ultracentrifugation and examined for antilipoidal and antitreponemal antibodies of the IgM and IgG classes.

Sera from the patients with late syphilis always showed persistent concentrations of antilipoidal IgM and IgG and of antitreponemal IgG but never yielded detectable concentrations of antitreponemal IgM. When treated, these patients' antibody titres did not decline. Patients with secondary or latent syphilis also showed this serological picture after treatment but only transiently; their antibody titres continued to decline in a way which clearly distinguished them from the cases of late syphilis.

It is suggested that patients whose sera persist in showing the stable pattern described may develop late symptomatic syphilis.

Introduction

The immunological responses at various stages of syphilis have been described by several workers. Aho[1] examined sera from patients with primary, secondary, latent, late, and congenital syphilis for antilipoidal antibodies separated into 19S and 7S fractions. Some sera from primary cases contained detectable antibodies in the 19S fraction only whereas others reacted with both 19S and 7S fractions. Patients with secondary or early latent syphilis usually showed higher titres in the 7S fraction. Sera from patients with late syphilis had a predominance of 19S antibodies. Julian *et al*[2] examined sera from cases of early syphilis for lipoidal antibodies and found activity in both 19S and 7S fractions. Sometimes there was reactivity in only the 7S fraction, but in no instance did they find reactivity in the 19S fraction alone.

Studies of the presence of specific antitreponemal IgM showed that such antibodies may be present in latent and late syphilis even after treatment.[3-9] In contrast, Duncan and Kuhn,[10] in a similar study using chimpanzees, found that production of specific antitreponemal IgM ceased spontaneously in 10 out of 24 animals who had received no treatment, and Shannon and Booth[11] failed to demonstrate antitreponemal IgM reactivity in sera from patients with late syphilis.

In the present study, we examined sera from cases of late symptomatic syphilis for the presence of IgM and IgG class antibodies against both lipoidal (A-L) and treponemal (A-T) antigens. These data, together with Venereal Disease Research Laboratory (VDRL) slide test results, were correlated with the clinical diagnosis to define the course of the immune response in late syphilis.

Patients and methods

SYPHILITIC SERA

One thousand three hundred and fifty specimens of sera from patients at various stages of the disease were selected from specimens taken at routine examination at the Bristol Public Health Laboratory. All the sera gave positive results in both the fluorescent treponemal antibody absorption (FTA-ABS) IgG test and the *Treponema pallidum* haemagglutination assay (TPHA).

FRACTIONATION PROCEDURES

Density gradient ultracentrifugations were performed in a 12·5-37·5% discontinuous sucrose gradient[12] using 5-ml polycarbonate ultracentrifuge tubes. A sample of serum (0·4 ml) was placed on top of the sucrose gradient and the tubes were centrifuged at 100 000 × *g* for 18 hours. Ten equal fractions were collected and subjected to

Address for reprints: Mr R Shannon, Public Health Laboratory, Myrtle Road, Kingsdown, Bristol BS2 8EL

Received for publication 4 April 1980

TABLE I *The clinical histories of cases of late syphilis*

Case No.	*Age (years)*	*Sex*	*No of serum samples*	*Time span of serology (months)*	*Time treatment was started*		*Clinical diagnosis*
					Before first specimen	*After first specimen*	
1	51	M	8	50	28 years	1 month	Cardiovascular syphilis
2	51	M	8	18		2 months	Meningovascular syphilis
3	57	M	7	24		1 month	Tabes dorsalis
4	68	M	6	18		1 month	Cardiovascular syphilis
5	42	M	2	12	4 years		Cardiovascular and neurosyphilis
6	65	M	3	11	*	*	Cardiovascular syphilis
7	57	M	3	1	*	*	Meningovascular syphilis
8	51	M	3	5	*	*	Neurosyphilis
9	59	M	1		*	*	Neurosyphilis
10	66	F	2	3	*	*	Meningovascular syphilis
11	54	M	2	1	*	*	Neurosyphilis
12	52	M	3	9	*	*	Cardiovascular syphilis
13		M	2	1	*	*	Tabes dorsalis
14	62	M	7	46	†	†	Neurosyphilis
15	79	M	1		†	†	Tabes dorsalis
16	55	M	7	30	†	†	Neurosyphilis
17	85	F	1		†	†	Gummatous syphilis
18	79	F	2	4	†	†	Cardiovascular syphilis

* Untreated
† History of treatment not known

complement-fixation tests (CFT) and fluorescent treponemal antibody absorption (FTA-ABS) tests.

Anti-lipoidal antibodies were detected by a Maltaner cardiolipin antigen CFT (Wellcome Reagents). Antitreponemal antibodies were detected by both Reiter protein antigen CFT (B-D Mérieux Antigène Tréponemique Souche Reiter) and FTA-ABS tests,[13] using specific antihuman IgM and IgG conjugates (Wellcome Reagents).

Results

LATE SYPHILIS

Fractionation procedures carried out on 31 serum samples from 18 patients presenting with symptoms of late syphilis (table I) showed that in all cases these sera contained A-L IgM and IgG and A-T IgG but no detectable concentrations of A-T IgM. The extended serological histories, available on nine patients with late symptomatic syphilis (cases 1-6, 12, 14, and 16), showed that there was little or no decrease in antibody titres whether they were treated or not. An example of the serological history of a case of late syphilis (case 1) is given in table II. From this, it can be seen that 15 months after treatment the titre of the A-T IgG had not fallen, the titres of the A-L IgM and IgG had fallen only moderately, and the VDRL titre remained constant within experimental variation.

The results of fractionation studies on a further 1319 serum samples were examined to determine whether the presence of A-L IgM and IgG and A-T IgG, in the absence of A-T IgM, was unique to patients with late clinical syphilis. This pattern was found at some stage of their immunological history in sera from 11 patients with treated secondary, from six with latent, and from three with adult congenital syphilis.

TABLE II *Immunological history of a patient (case 1) with late symptomatic syphilis**

Time (months)	*VDRL titre¶*	*Cardiolipin CFT titres*		*RPCFT titres*		*FTA-ABS-IgM test*
		IgM	*IgG*	*IgM*	*IgG*	
0†	8	49	22	0	28	0
2‡	4	49	21	0	23	0
4§	8					
6§	16					
10	8	51	15	0	22	0
14	8	22	13	0	24	0
17	16	12	12	0	26	0
50§	8					

* This patient had a history of syphilis in 1946, when he was treated with arsenic and bismuth. In 1974 he developed an aortic aneurysm. Eight serum samples were received from this patient.
† Time 0 represents serology on presentation.
‡ Treatment was started immediately after this sample had been taken.
§ Insufficient serum for fractionation procedure.
¶ Reciprocal of the dilution.
CFT = Complement-fixation test
RPCFT = Reiter protein complement-fixation test

SECONDARY SYPHILIS

Twenty-seven serum samples from 11 patients who had been treated for secondary syphilis showed the pattern A-L IgM and IgG and A-T IgG but no A-T IgM. Sera from all these patients contained A-T IgM and the other three types of antibody at the time of diagnosis before treatment was given.

TABLE III *Immunological history of a patient with secondary syphilis*

		Cardiolipin CFT titres		RPCFT titres		
Time (months)	*VDRL titre*‡	*IgM*	*IgG*	*IgM*	*IgG*	*FTA-ABS-IgM test*
0*†	32	94	222	26	65	+
2	8	17	19	0	18	0
3	4	13	19	0	8	0
5	2	6	2	0	5	0
9	0	4	0	0	4	0

* Time 0 represents serology on presentation.
† Treatment was started immediately after this sample had been taken.
‡ Reciprocal of the dilution.
+ Positive

After treatment, the A-T IgM titre fell below detectable levels, thus giving the serological pattern found in the 18 cases of late syphilis. Thereafter, however, the antibody titres declined and A-L IgG was usually the next to fall below detectable levels. This led to the appearance of the serological pattern (presence of A-L IgM and A-T IgG) that was observed in patients treated successfully.[11] The serological response of a typical case of secondary syphilis is given in table III. In secondary syphilis antibody concentrations fall rapidly after treatment unlike in late syphilis, where they show little or no reduction (table II). Secondary syphilis may therefore resemble late syphilis serologically for a time (usually not for more than three months) during this period; however, the titres continue to fall.

LATENT SYPHILIS

Examination of the immune response of patients diagnosed as having latent syphilis showed that only six, at some stage, had the A-L IgM and IgG and A-T IgG pattern. These patients fell into two groups: three had detectable concentrations of antitreponemal IgM at some stage but the other three had none. Two of the patients who presented with detectable concentrations of A-T IgM showed the serological pattern of treated secondary syphilis—that is, as their immune response declined after treatment, it transiently resembled that of late syphilis (table IV). The other patient, in whom concentrations of A-T IgM soon became undetectable, however, showed minimal decrease in the titres of the other three types of antibody despite treatment (table V). This patient may be considered a treatment failure and so liable to develop late symptomatic syphilis.

The other three cases of latent syphilis with no A-T IgM antibodies (table VI) serologically resembled cases of late syphilis. As in the example, however, their antibody concentrations fell markedly after treatment in contrast to cases of late syphilis, but the fall in titre was considerably slower than in the cases of secondary syphilis. A fourfold decrease in VDRL test titre took approximately two months in cases of treated secondary syphilis and eight months in these cases.

TABLE IV *Immunological history of a patient with latent syphilis presenting with a positive FTA-ABS-IgM test result*

		Cardiolipin CFT titres		RPCFT titres		
Time (months)	*VDRL titre*‡	*IgM*	*IgG*	*IgM*	*IgG*	*FTA-ABS-IgM test*
0*†	16	225	21	13	50	+
1†	8	267	21	10	38	+
6†	2	119	9	0	2	±
16	0	8	0	0	2	0
23	0	0	0	0	0	0

* Time 0 represents serology on presentation.
† Treatment was started immediately after these samples had been taken.
‡ Reciprocal of the dilution.
+ Positive; ± weakly positive

TABLE V *Immunological history of a patient with latent syphilis presenting with a positive FTA-ABS-IgM test result and possibly developing late syphilis*

		Cardiolipin CFT titres		RPCFT titres		
Time (months)	*VDRL titre*‡	*IgM*	*IgG*	*IgM*	*IgG*	*FTA-ABS-IgM test*
0*	32	59	94	13	72	+
1†	32	79	129	0	90	+
3†	128	54	146	0	162	+
11	16	48	74	0	16	0
12	8	28	30	0	45	0
16	16	12	16	0	32	0
17	32	12	24	0	32	0

* Time 0 represents serology on presentation.
† Treatment was started immediately after these samples had been taken.
‡ Reciprocal of the dilution.
+ Positive

TABLE VI *Immunological history of a patient with syphilis presenting with a negative FTA-ABS-IgM test result*

		Cardiolipin CFT titres		RPCFT titres		
Time (months)	*VDRL titre*‡	*IgM*	*IgG*	*IgM*	*IgG*	*FTA-ABS-IgM test*
0*†	16	28	46	0	93	0
4	8	25	45	0	24	0
7	4	16	21	0	25	0
26	+	3	0	0	6	0

* Time 0 represents serology on presentation.
† Treatment was started immediately after this sample had been taken.
‡ Reciprocal of the dilution.
+ Positive (undiluted)

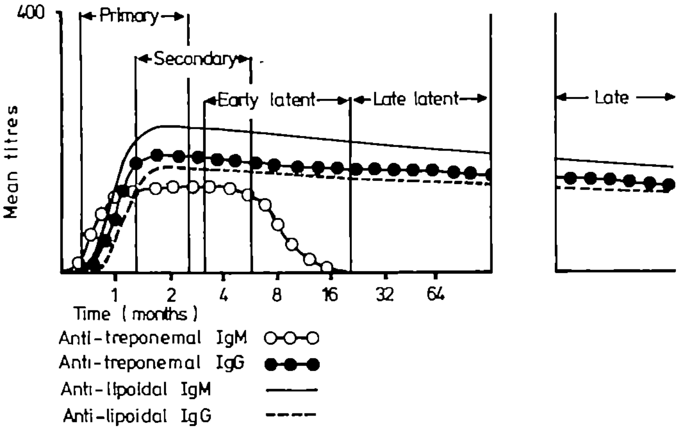


FIGURE *Immune responses in untreated syphilis*

CONGENITAL SYPHILIS

Adult congenital syphilis was diagnosed in three patients. The serological pattern of these patients was the same as that which we have associated with late syphilis. Clinical details were not available for one of the three cases, but the other two presented with signs and symptoms of late syphilis.

One patient presented with an unusual rash and could not therefore be included in any of the above categories. Serum from this patient was shown initially to contain both A-L IgM and IgG and A-T IgM and IgG antibodies, but after treatment the A-T IgM concentration fell below detectable levels. The A-L IgM and IgG and A-T IgG concentration thus remained unaltered 18 months after treatment. The serological pattern of this patient was identical to that seen in the case of latent syphilis described previously and illustrated in table V.

Discussion

Although examination of sera for A-T IgM antibodies (usually by the FTA-ABS-IgM test) may provide a useful indication of the active state of the disease in primary or secondary cases of syphilis, it would appear to be unreliable in patients at later stages.

We have shown above that 18 patients, diagnosed on clinical evidence as cases of late syphilis had no detectable concentrations of A-T IgM in their sera taken before or after treatment. Of six patients diagnosed as cases of latent syphilis three had A-T IgM in their sera taken before treatment but three did not. In contrast, patients presenting with untreated secondary syphilis invariably had A-T IgM present in their sera.

The results suggest that during the course of untreated syphilis A-T IgM production ceases, generally during the latent period between the end of secondary symptoms and the onset of late symptoms. These findings are consistent with those of Wilkinson and Rodin,[8] who found that the number of positive FTA-ABS-IgM test results was considerably reduced by the time patients had progressed to the late latent stage of the disease. Unfortunately, no patients with untreated late symptomatic syphilis attended during their survey. O'Neill[9] also agreed that some cases of latent syphilis may present with a negative FTA-ABS-IgM test result.

Patients who have reached the late symptomatic stage of the disease, whether treated or not, lose the ability to produce A-T IgM while retaining high concentrations of A-L IgM and IgG and A-T IgG antibodies. O'Neill[9] suggested that cases of late syphilis, whether treated within the preceding five years or untreated, should be FTA-ABS-IgM-positive. Our findings do not support this opinion.

It is also apparent that successful treatment of secondary syphilis produces a cessation of A-T IgM production, and for a short period the serological pattern may mimic that of late syphilis (table III), but antibody concentrations continue to fall. The next immunoglobulin to become undetectable is A-L IgG, leaving A-L IgM and A-T IgG, a combination of immunoglobulins found to be typical of successfully treated syphilis.[11] Cases of treated latent syphilis showed a similar decline in antibody concentrations although at a lower rate than that seen in cases of secondary syphilis (tables IV and VI).

We therefore suggest that, because sera from treated cases of secondary and latent syphilis produce the serological pattern that we associate with late syphilis for only a short period, this is unlikely to be confused with the persistent response that is typical of late syphilis. The patients—one diagnosed as having latent syphilis (table V) and the other presenting with an unusual rash—who showed no such fall in antibody concentrations after treatment may be liable to develop late symptomatic syphilis in the future.

The patterns of the immune response in untreated syphilis are illustrated in the figure, where it can be seen that if an infection with *Treponema pallidum* is allowed to run its course untreated then some time after secondary signs have disappeared and the disease becomes latent the A-T IgM response declines. The latent stage can therefore be redefined as early latent—when A-T IgM antibody is still present—and late latent—when A-T IgM antibody has disappeared. Treatment of patients with latent syphilis usually produces a reduction in antibody concentrations to give the A-L IgM and A-T IgG picture that is typical of successful treatment. When the patient enters the late symptomatic stage of the disease the A-L IgM and IgG and A-T IgG responses remain typically raised and are unaffected by treatment. The persistence of the immune response in late syphilis may be due either to a reservoir of treponemes in locations inaccessible to antibiotics or to their presence in a non-dividing vegetative state,[14] and thus late syphilis may not respond serologically to treatment. Thus, a patient whose serum persistently produces the serological pattern of A-L IgM and IgG, and A-T IgG reactivity, may be considered a candidate for the development of late symptomatic syphilis.

We are indebted not only to Dr H R Cayton, director (retired) of the Bristol Public Health Laboratory, but also to Dr A E Jephcott, his successor, for granting permission and facilities to carry out this work and for helpful advice and criticism. We are also grateful to the members of staff of the Public Health Laboratory and various STD clinics, without whose help this work could not have been done.

References

1. Aho K. Studies of syphilitic antibodies. 1 Antilipoidal antibodies in various stages of syphilis. *Br J Vener Dis* 1967;**43**:259-63.
2. Julian AJ, Logan LC, Norins LC. Early syphilis: immunoglobulins reactive in immunofluorescence and other serological tests. *J Immunol* 1969;**102**:1250-9.
3. Atwood WG, Miller JL. Fluorescent treponemal antibodies in fractionated syphilitic sera. *Arch Dermatol* 1969;**100**:763-9.
4. Atwood WG, Miller JD. The immunoglobulin class of fluorescent treponemal antibodies in syphilis. *Int J Dermatol* 1970;**9**:259-66.
5. Logan LC, Norins LC, Atwood WG, Miller JL. Treated late syphilis: immunoglobulin class of antibodies reactive with *Treponema pallidum*. *J Invest Dermatol* 1969;**53**:300-1.
6. Julian AJ, Logan LC, Norins LC, Scotti AT. Latent syphilis: Immunoglobulins reactive in immunofluorescence and other serological tests. *Infect Immun* 1971;**3**:559-61.
7. O'Neill P, Nicol CS. IgM class anti-treponemal antibody in treated and untreated syphilis. *Br J Vener Dis* 1972;**48**:460-3.
8. Wilkinson AE, Rodin P. IgM-FTA test in syphilis in adults: its relation to clinical findings. *Br J Vener Dis* 1976;**52**:219-23.
9. O'Neill P. A new look at the serology of treponemal disease. *Br J Vener Dis* 1976;**52**:296-9.
10. Duncan WP, Kuhn USG. Treponemal IgG and IgM response in experimentally infected chimpanzees. *Br J Vener Dis* 1974;**50**:257-63.
11. Shannon R, Booth SD. The pattern of immunological responses at various stages of syphilis. *Br J Vener Dis* 1977;**53**:281-6.
12. Vesikari T, Veheri A. Rubella: a method for rapid diagnosis of a recent infection by demonstration of the IgM antibodies. *Br Med J* 1968;i:221-3.
13. Wilkinson AE. Laboratory diagnosis of venereal disease. *Monograph Series* No I. London: Public Health Laboratory Service, 1972;18-24.
14. Collart P, Borel LJ, Durel P. Significance of spiral organisms found after treatment in late human and experimental syphilis. *Br J Vener Dis* 1964;**40**:81-9.

Br J Vener Dis 1980;**56**:377-80

Value of serological diagnosis in congenital syphilis

Report of nine cases

M V BOROBIO, M C NOGALES, AND J C PALOMARES

From the Department of Microbiology and Preventive Medicine, University Hospital, Seville, Spain

SUMMARY The diagnosis of congenital syphilis is difficult since it depends mainly on the results of serological tests. The results of five serological tests (three specific and two non-specific) in nine neonates with congenital syphilis are compared with those obtained in three with passively acquired antibodies. It appears that the serological diagnosis of congenital syphilis must be based on the finding of specific neonatal antibodies in cord serum, which give positive results to the fluorescent treponemal antibody absorption test for immunoglobulin M, together with high titres of total IgM and negative results to latex tests. The non-specific tests are useful for confirming the efficacy of treatment.

The mean number of cases of congenital syphilis in Seville is 0·81/1000 live births.

Introduction

Congenital syphilis reached a low level during the late 1950s. With the increase in infectious syphilis in the adult population during the 1960s, however, the incidence of early congenital syphilis in infants under 1 year of age increased in many parts of the world. In the United States 132 cases were reported in 1969 and 144 in 1977[1]; the number then seemed to have stabilised, with 107 cases in 1978. During the period from January to March 1979 the number of cases fell by 16% from that reported in the same period the previous year.[2] The evolution in England has been similar. Cases of syphilis are rare in Sweden.[3] In Spain there are no statistics on the incidence of this disease.

The diagnosis of congenital syphilis presents a considerable problem, since it depends mainly on the results of serological tests and also because most syphilitic neonates are asymptomatic at birth.

All the standard serological tests for syphilis depend on responses involving IgG and IgM antibodies.[4] This makes their interpretation in neonates extremely difficult as the IgG antibody found in the serum of neonates is largely passively acquired through the placenta[5] and does not represent the infant's own response. In 1968 Scotti and Logan[4] described the fluorescent treponemal antibody absorption test for antitreponemal IgM antibodies (IgM-FTA-ABS). In theory, any antitreponemal IgM found in the serum of the neonate would be expected to have been produced by the baby in response to the *T pallidum* present in its tissues.

The aims of this work were: (a) to determine the incidence of congenital syphilis in the population of Seville; and (b) to evaluate the different serological tests in the diagnosis of congenital syphilis.

Over a period of three years we have studied nine cases of either symptomatic or asymptomatic early congenital syphilis using the IgM-FTA-ABS test together with three other specific and two non-specific tests for syphilis. We have compared these results with those obtained in three neonates with passively acquired antibodies. We have followed most cases for several months and have monitored the treatment and evolution of the disease.

Patients and methods

STUDY POPULATION

The sera of all antenatal patients attending the hospital were screened by the Venereal Disease Research Laboratory (VDRL) test; sera giving positive results were confirmed by the FTA-ABS test. Most women arriving to give birth in our hospital have never attended this hospital for antenatal care. Over a period of three years 4000 VDRL tests were performed and 6% gave positive results. Of these 6% (240) only 0·3% (12) gave positive results by the FTA-ABS-IgG and FTA-ABS-IgM tests, a further eight gave positive results by the FTA-ABS-IgG test but negative results by the FTA-ABS-IgM test.

Address for reprints: Dr M V Borobio, Department of Microbiology and Preventive Medicine, University Hospital, University of Seville School of Medicine, Seville 9, Spain

Received for publication 25 February 1980

TABLE *Results of serological tests in nine infants with congenital syphilis (cases 1-9) and in three with passively acquired antibodies (cases 10-12)*

			Results of serological tests							
									FTA-ABS‡	
Case Nos	*Sex*	*Age (days)*	*Total IgM (g/l)**	*VDRL (titre)*	*RST (titre)*	*Latex (1/125 dilution)*	*TPI†*	*TPHA (titre)*	*IgG*	*IgM*
1	M	1	2·05	1/128	1/128	–	+	1/1640	2+	2+
		30	1·15	1/128	1/128	–	+	1/640	2+	2+
		90	1·06	1/16	1/16	–	+	1/640	2+	2+
		150	1·00	1/4	1/4	–	+	1/640	2+	–
1	F	1	2·15	–	–	–	+	–	–	–
		30	1·84	–	–	–	+	–	–	–
		150	1·05	–	–	–	+	–	–	–
2	F	1	3·00	1/64	1/64	–	+	1/640	2+	2+
		120	1·55	1/64	1/64	–	+	1/640	2+	2+
3	F	1	2·15	–	–	–	+	1/2560	4+	4+
		120	1·06	–	–	–	+	1/2560	4+	–
4	M	1	3·50	1/256	1/256	–	+	1/2560	4+	3+
		30	2	1/32	1/32	–	+	1/2560	4+	3+
		60	1	1/8	1/8	–	+	1/2560	4+	3+
5	F	150	1·4	1/16	1/16	–	+	1/640	2+	2+
		180	1·9	1/16	1/16	–	+	1/640	2+	2+
		990	1·4	1/8	1/8	–	+	1/640	2+	1+
6	M	1	3·2	1/2	1/2	–	+	1/320	3+	2+
		90	2·4	–	–	–	+	1/80	2+	2+
7	M	1	0·3	1/4	1/4	–	+	1/320	3+	2+
8	F	5	4·7	1/2	1/2	–	+	1/1280	4+	4+
9	M	1	2·06	1/64	1/32	–	+	1/1200	4+	3+
10	M	1	0·7	–	–	–	+	1/320	2+	–
		74	0·7	–	–	–	+	1/320	2+	–
		88	0·66	–	–	–	+	1/320	2+	–
11	F	1	0·66	+	+	–	+	1/320	+	–
		97	0·6	–	–	–	+	1/160	+	–
12	F	1	0·32	–	–	–	+	1/160	+	–

*Normal values of IgM in neonates are between 0·16 and 0·2 g/l
†TPI test: + = 100% immobilisation
‡FTA-ABS test: 1+, 2+, 3+, 4+ = increasing intensity of fluorescence
+ Positive – negative
Conversion: SI to traditional units — immunoglobulin concentration 1 g/l ≈ 100 mg/100 ml

The 12 cases reported here were detected by routine screening of the infants' mothers. Nine babies had congenital syphilis and three had passively acquired antibodies. All were followed for 5-6 months. The parents and siblings were also studied.

SEROLOGICAL TESTS

Non-specific and specific serological tests were carried out.

Non-specific. These included: estimation of total IgM in serum[6]; the latex test (the serum was tested at a dilution of 1/25)[7]; the VDRL qualitative and quantitative test[8]; and the qualitative and quantitative reagin screen test (RST).[9]

Specific. These included: the FTA-ABS test[10]; the FTA-ABS-IgM test[4]; the *T pallidum* haemagglutination assay (TPHA)[11 12] (qualitative and quantitative determinations using microtitre plates); and the *T pallidum* immobilisation (TPI) test.[13]

Three standard sera giving positive, weakly positive, and negative results were included in each batch of tests as controls.

TREATMENT

Pregnant women were treated according to the Willcox schedule[14] using procaine penicillin 0·6 megaunits/day for eight days (total dose 4·8 megaunits).

Syphilitic neonates were treated with procaine penicillin G 50 000-100 000 units/kg body weight daily for at least 15 days.[15]

Results

The results of the serological tests are shown in the table.

CASE 1

This was a twin birth, in which only the boy had clinical evidence of congenital syphilis. He was born at term following an uncomplicated twin delivery. Height and weight were below the tenth percentile. The infant was pale; the liver was palpable at 4 cm and the spleen at 1 cm below the costal margins. A roentgenogram showed periosteal new bone formation on several of the ribs and on the proximal area of the humerus. Additional films showed thickened periosteal new bone in the extremities. His total IgM was 2·05 g/l (205 mg/100 ml) and all serological test results were strongly positive. The tests were repeated three and five months later. Only the FTA-ABS-IgM and VDRL-RST tests were modified after treatment, and at the fifth month the first gave negative results while the total IgM was normal.

The girl showed no clinical evidence of congenital syphilis and all serological tests gave negative results except the TPI; total IgM concentration was raised.

The discrepancy between the serological results of the girl and that of her twin was because the boy was affected through the placenta and the girl was not as she had a different placenta.

CASE 2

This was a girl, who had clinical evidence of congenital syphilis at birth. At eight hours after birth she had a swollen, tender, crepitant left elbow and limited abduction of the left hip. Roentgenograms showed that the left hip was dislocated with evidence of destruction in the proximal metaphysis and periosteal reaction. All the tests performed gave positive results with high values of total IgM. Four months later the tests produced similar results except for total IgM, which fell from 3 g/l to 1·55 g/l (300 mg/100 ml to 155 mg/100 ml).

CASE 3

This was a girl, who showed no clinical evidence of congenital syphilis at birth. The results of the non-specific tests were negative and those of the specific tests were positive with raised total IgM. Six months later the total IgM concentration was normal and the FTA-ABS-IgM test gave a negative result.

CASE 4

This was neonate, who did not show clinical evidence of congenital syphilis. The tests all gave positive results with high concentrations of total IgM.

CASE 5

This was a 5-month-old girl, in whom the only clinical symptom was a slight tibial osteitis. All tests gave strongly positive results, which remained positive until six months later.

CASE 6

This was a 2-week-old infant, who was admitted to hospital because of anaemia. He had a hypopigmented rash on the back and neck, which later spread over the body to the hands and feet. The lesions included blisters, vesicles, and wrinkled peeled areas. His haemoglobin was 6·6 g/dl. Roentgenograms showed a solitary abnormality of the right tibia with resorption of the cortex. The parents were diagnosed as having syphilis and were treated at the beginning of the mother's pregnancy.

CASES 7 AND 8

These were two polymalformed neonates, who died during the first month of life. The second child had some perianal condylomata which harboured numerous treponemes.

CASE 9

This was a neonate, whose mother had been diagnosed as having syphilis late in pregnancy.

Latex tests gave negative results in all the nine cases studied. Serological tests were carried out on samples of cerebrospinal fluid from cases 1, 3, 4, and 9, and all of them gave negative results.

PASSIVE ANTIBODY TRANSFER

Three cases of passive antibody transfer through the placenta are summarised in the table. The results of all the tests measuring IgG were positive and those of tests measuring IgM were negative while total IgM values were normal.

BIRTH STATISTICS

An analysis of the birth statistics at the University Hospital of Seville over a three-year period showed that out of 11 110 live births nine babies had neonatal syphilis, an incidence of 0·81/1000 live births.

Discussion

Since its original description in 1968, the usefulness and accuracy of the monospecific FTA-ABS-IgM test for congenital syphilis has been assessed by several workers.[16] Some have found doubtful or negative results in syphilitic babies.[17 18] False-positive results have also been reported.[19]

The nine cases of congenital syphilis we studied had positive results to the FTA-ABS-IgM tests and these remained positive as long as the total serum IgM was higher than normal. Furthermore, we were unable to detect IgM specific antibodies in the three patients with passively acquired antibodies who showed normal values of total IgM. These findings indicate that we did not encounter either false-negative or false-positive results for the FTA-ABS-IgM test.

Recent studies suggest that the FTA-ABS-IgM test may detect IgM directed against maternal IgG rather than against *T pallidum*.[21 22] This anti-antibody appears to be similar to the rheumatoid factor found in many adults. Some workers have reported false-positive tests due to adult rheumatoid factors.[12 20 22 23] Because of the possibility that this rheumatoid factor might lead to an apparently positive FTA-ABS-IgM test, the latex test must be carried out to exclude false-positive results[7 24]; this test gave negative results for all of the nine babies with congenital syphilis we studied.

When the cases of congenital syphilis are compared with those of passively acquired antibodies, we did not find any differences in the results of the FTA-ABS test, TPHA, and TPI test because these tests depend only on maternal antibody titres. Differences were found in the total IgM titres—which are normal in cases of passive transfer and very high in syphilitic babies—and in the FTA-ABS-IgM test, which gave positive results only for the syphilitic babies. In the non-specific tests higher titres were obtained in the syphilitic babies than in their mothers and lower titres in the babies with passive transfer than in their mothers.

We conclude that serological diagnosis of congenital syphilis must be based on the demonstration of specific neonatal antibodies in the cord serum. As these antibodies belong to the IgM fraction, high titres of total IgM—together with positive FTA-ABS-IgM test results and negative latex test results—are the findings needed to make a serological diagnosis of congenital syphilis.

The results of non-specific tests are useful during follow-up after treatment to confirm its efficacy.

References

1. Center for Disease Control. Reported morbidity and mortality in the United States. *Morbidity and Mortality Weekly Report* 1978;**27**:346-7.
2. Center for Disease Control. Congenital syphilis—United States 1978-1979. *Morbidity and Mortality Weekly Report*. 1979;**28**: 433-4.
3. Meheus AZ. Epidemiologie des maladies à transmission sexuelle (MTS) dans le monde. In: Garcia-Pérez A, Perea EJ, eds. *Enfermedades de Transmisión Sexual*. Seville: Universidad de Sevilla, 1979.
4. Scotti AT, Logan L. A specific IgM antibody test in neonatal congenital syphilis. *J Pediatr* 1968;**73**:242-3.
5. Alford, CA, Polt SA, Cassady GE, Straumfjord JV, Remington JS. M-fluorescent treponemal antibody in the diagnosis of congenital syphilis. *N Engl J Med* 1969;**280**:1086-91.
6. Laurell CB. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Ann Clin Biochem* 1966;**15**:45-9.
7. Wilkinson AE, Rodin P. IgM-FTA test in syphilis in adults. Its relation to clinical findings. *Br J Vener Dis* 1976;**52**:519-23.
8. US Department of Health, Education and Welfare. *Serologic Tests for Syphilis*, PHS Publication No 411. Washington DC: US Government Printing Office, 1964.
9. *Diagnostic Agents for Syphilis used in Serologic Tests*, Pearl River, NY: Lederle Laboratories, 1962.
10. Hunter EF, Deacon WE, Meyer PC. An improved FTA test for syphilis, the absorption procedure (FTA-ABS). *Publ Health Rep* 1964;**79**:410-2.
11. Johnston NA. *Treponema pallidum* haemagglutination test for syphilis. Evaluation of a modified micro-method. *Br J Vener Dis* 1972;**48**:474-8.
12. Tomizawa T, Kasamatsu S, Yamaya S. Usefulness of the hemagglutination test using *Treponema pallidum* antigen (TPHA) for the serodiagnosis of syphilis. *Jap J Med Sci Biol* 1969;**22**:341-50.
13. Nelson RA, Mayer MM. Immobilization of *Treponema pallidum* in vitro by antibody produced in syphilitic infection. *J Exp Med* 1949;**39**:369-93.
14. Willcox RR. *Textbook of Venereal Diseases and Treponematoses*. London: William Heinemann, 1964.
15. Garcia-Perez A. Sifilis: tratamiento. In: Garcia-Pérez A, Perea EJ, eds. *Enfermedades de Transmisión Sexual*. Seville: Universidad de Sevilla. 1979.
16. Rosen EU, Richardson NJ. A reappraisal of the value of IgM fluorescent treponemal antibody absorption test in the diagnosis of congenital syphilis. *J Pediatr* 1975;**87**:38-42.
17. Alford CA. Immunglobulin determinations in the diagnosis of fetal infection. *Pediatr Clin North Am* 1971;**18**:99-101.
18. Johnston NA. Neonatal congenital syphilis. Diagnosis by the absorbed fluorescent treponemal antibody (IgM) test. *Br J Vener Dis* 1972;**48**:464-57.
19. Sepetjian F, Tissot Guerraz F, Monier JC, Nivelon JL, Thivolet J. Investigation of a specific IgM antibody test in neonatal congenital syphilis. *Br J Vener Dis* 1970;**46**:18-20.
20. Reimer CB, Black CM, Phillips DJ, *et al*. The specificity of fetal IgM: antibody or anti-antibody? *Ann NY Acad Sci* 1975;**254**:77-93.
21. Speiser P. New aspects of immunogenetic relationships between child and mother. I Children produce antibodies against their mother's antigen. *Ann Pediatr* 1966;**207**:20-35.
22. Wilkinson AE, Rayner CFA. Studies on the fluorescent treponemal antibody (FTA) test. *Br J Vener Dis* 1966;**42**:8-15.
23. Notkins AL. Infectious virus-antibody complexes: interaction with anti-immunoglobulins, complement and rheumatoid factor. *J Exp Med* 1971;**134**:415-505.

Br J Vener Dis 1980;56:381-6

Lack of serological evidence for venereal spirochaetosis in wild Victorian rabbits and the susceptibility of laboratory rabbits to Treponema paraluis-cuniculi

S R GRAVES,* J W EDMONDS,† AND ROSAMOND C H SHEPHERD†

*From the *Microbiology Department, Monash University, Clayton, and the †Keith Turnbull Research Institute, Frankston, Victoria, Australia*

SUMMARY Sera from 608 wild rabbits were examined using serological tests for syphilis as an indicator of infection with *Treponema paraluis-cuniculi.* Only eight sera gave positive or weakly positive results in the rapid plasma reagin (RPR) test, and none of these eight sera gave positive results in the *Treponema pallidum* haemagglutination assay (TPHA). Thus, it appears that wild rabbit populations in Victoria, Australia, are not naturally infected with *T paraluis-cuniculi.*

Normal Australian laboratory rabbits however were readily infected with *T paraluis-cuniculi,* either by intradermal or intratesticular inoculation or by the venereal route. In the latter case, treponeme-containing lesions developed after about five months' cohabitation with infected mates. The disease was successfully transmitted from male to female and from female to male rabbits by the venereal route. In most cases infected rabbits became RPR-positive (17/19 rabbits) and in all cases TPHA-positive (19/19), indicating that serological tests for syphilis can be used to screen rabbits for this disease.

Introduction

Rabbit venereal spirochaetosis is a sexually transmitted bacterial disease caused by *Treponema paraluis-cuniculi.* It occurs in European wild and laboratory rabbits, *Oryctolagus cuniculus* (L), in laboratory rabbits in North America, and probably in European hares,[1] *Lepus europaeus* (Pallus), but it has not been reported in Australia.

Rabbits are not native to Australia. They were introduced in the late eighteenth and nineteenth century as a result of European colonisation of the continent and became well established as a feral animal in Victoria during the 1860s. Although the predominant progenitors were wild-type rabbits, domestic rabbits contributed to the gene pool in several locations.[2]

T paraluis-cuniculi is closely related, morphologically and antigenically, to *Treponema pallidum,* the causative agent of syphilis in man.[3] Rabbits infected with *T paraluis-cuniculi* develop antibodies that can be detected using standard serological tests for syphilis.[4] In this study sera taken from wild rabbits from various parts of Victoria were tested for treponemal antibodies using both a non-specific test for syphilis, the rapid plasma reagin (RPR) test, and a highly specific test for treponemal infection, the *T pallidum* haemagglutination assay (TPHA).

Australian laboratory rabbits were also tested for their susceptibility to *T paraluis-cuniculi* by intradermal, intratesticular, intraurethral, and venereal transmission, and the infections were followed clinically, bacteriologically, and serologically.

Materials and methods

Wild rabbits were collected from 19 areas* of Victoria.[5] Blood was taken via the marginal ear vein from live rabbits and from the jugular vein or heart of shot rabbits. The serum was collected and stored frozen until required.

Address for reprints: Dr S R Graves, Microbiology Department, Monash University Medical School, Alfred Hospital, Prahran, Victoria 3181, Australia

Received for publication 12 March 1980

---

*Details of the areas surveyed can be obtained from the authors on request.

SEROLOGICAL TESTS

The RPR test was carried out according to the manufacturer's instructions (Commonwealth Serum Laboratories, Melbourne, Australia). Results were recorded as strongly positive, weakly positive, or negative.

The TPHA was performed at Fairfield Hospital, Melbourne, or at Monash University, using the kit produced by Fujizoki Pharmaceutical Company Ltd. The manufacturer's instructions were followed. Only titres greater than 1/80 were considered positive and therefore indicative of past or current treponemal infection. Known positive and negative control sera were included in each test series.

INFECTION WITH *T PARALUIS-CUNICULI* (LABORATORY RABBITS)

Intradermal inoculation

T paraluis-cuniculi (strain 8816) was obtained from the Center for Disease Control, Atlanta, Georgia, USA, by courtesy of Dr A Balows. It was sent to Australia in the frozen orchitic testis of a rabbit that had been infected intratesticularly. It has subsequently been maintained by intratesticular inoculation in rabbits or stored frozen (−70°C) in an equal mixture of 30% glycerol in isotonic saline (prereduced) and *T pallidum* maintenance medium.[6] Male rabbits inoculated intradermally on the shaved back or intratesticularly were kept in individual cages at 16-19°C with antibiotic-free food and water in unlimited quantities.

Venereal transmission

In the venereal transmission experiments rabbits were kept in spacious open pens (completely surrounded with wire-mesh) at the Keith Turnbull Research Institute, Frankston, Victoria. They were fed antibiotic-free food and water and occasionally grass.

In the experiment to test male-to-female venereal transmission of *T paraluis-cuniculi* one experimentally infected male rabbit (No 82673) was enclosed with four normal female rabbits. The male rabbit had been infected intratesticularly, intraurethrally, and under the prepuce (Table I) with a concentrated (approximately 10^7/ml) preparation of *T paraluis-cuniculi* obtained from frozen stocks in glycerol/medium. It was not possible to place more than one male rabbit in the pen with the females because of fighting between the males.

In the experiment to test female-to-male venereal transmission of *T paraluis-cuniculi* one normal male rabbit (No 83324) was enclosed with the four female rabbits above after they had all become infected, as shown by the presence of treponeme-containing genital lesions. The previous male rabbit (No 82673) was removed at this stage.

EXAMINATION FOR LESIONS

Rabbits were examined at regular intervals for genital lesions. Genital scrapings were taken by gently abrading the surface of the lesion with a scalpel blade and adding drops of sterile saline (approximately 0·5 ml) from a Pasteur pipette. The exudate-saline mixture was collected in the same Pasteur pipette and examined by darkfield microscopy for the presence of treponemes. Blood samples were taken from the marginal ear vein and the serum examined for antibodies by the RPR test and the TPHA.

Results

WILD RABBITS

Of the 608 rabbit sera examined, only eight gave positive results in the RPR test, and of these four were weak reactions. These eight sera all gave negative results to the TPHA.

TABLE I *Treponema paraluis-cuniculi infection in male laboratory rabbits with site of inoculation*

	Rabbit No and site of inoculation				
	580	*581*	*582*	*607*	*82673*
Response to infection	*Testes*	*Testes*	*Prepuce/ urethra**	*Testes/ prepuce/ urethra*	*Testes/ prepuce/ urethra*
Orchitis	Yes	Yes	No	Yes	No
Transient granular surface lesions on testes (treponemes present)	No	Yes	No	No	No
Erythematous lesions on prepuce and external genitalia (treponemes present)	No	Yes	Yes	No	Yes
Spontaneous healing of lesions	†	Yes	Yes	Yes	Yes
RPR test (days after infection)	†	+ + (75)	+ (195)	+ (35)	± (48)
TPHA titre (days after infection)	†	1/640 (137)	1/640 (138)	1/1280 (70)	1/1280 (120)

*Surface inoculation only; skin not broken
†Rabbit killed at height of orchitis to transfer bacterium
+ + Very strongly positive; + strongly positive; ± Weakly positive

One of us (RCHS), having examined thousands of wild rabbits throughout Victoria, has never seen genital lesions resembling those of *T paraluis-cuniculi* as manifested in the venereally infected laboratory rabbits in this study. We therefore conclude that this disease is not present in wild Victorian rabbits.

INFECTION WITH *T PARALUIS-CUNICULI* (LABORATORY RABBITS)

Intradermal inoculation

Ten male rabbits, each inoculated intradermally at one site on their shaved backs with 2 × 10^7 viable treponemes, usually developed lesions by day 6 after inoculation, although in one (No 17) they took 55 days to develop (table II). The early lesions appeared as small areas of erythema and induration in the skin, which gradually enlarged. These early lesions were very similar to early *T pallidum* lesions in rabbits.[3] They reached their maximum size (usually 5-9 mm diameter induration) at various times between 10 and 136 days after inoculation (table II) and then regressed at varying rates. In four of the rabbits the lesions disappeared between 55 and 70 days after inoculation; in another two at about 218 days and in another three the lesions were still present 365 days after inoculation, at the end of the observation period.

Both the RPR test and TPHA gave positive results 84 days after inoculation, indicating that the infection had stimulated both a specific and non-specific antibody response in the rabbits.

As the dermal lesions faded they became less indurated with a smaller zone of erythema. In most cases the lesion surface became granular and crumbled on gentle abrasion. In three rabbits (Nos 14, 21, and 23) secondary (or satellite) lesions formed around the original lesion 202, 149, and 246 days after inoculation. In all cases they were considerably smaller than the original lesion. These three rabbits still had their original lesion 365 days after inoculation and the development of secondary lesions probably represented a poor or slow development of immunity. In one rabbit the four satellite lesions healed before the end of the observation period whereas in the other two they were still present.

Other sites of inoculation

In this study two rabbits were inoculated intratesticularly, two intratesticularly, intraurethrally, and under the prepuce, and one intraurethrally and under the prepuce, care being taken not to pierce the skin in the latter case.

All five rabbits became infected and the four rabbits tested by the RPR test and TPHA developed antibodies (table I). The location of the lesions varied depending partly on the site of infection (table I), but in all cases the lesions healed spontaneously. Rabbits Nos 607 and 82673 were capable of producing normal offspring. The other three rabbits were not tested.

Male-to-female venereal transmission

Male rabbit No 82673 developed treponeme-positive genital lesions after intratesticular, intraurethral, and sub-preputial inoculation with viable *T paraluis-cuniculi* from frozen material. Immediately after inoculation he was placed in a pen with four normal serologically negative female rabbits (Nos 658, 91326, 91249, and 21271) to ascertain whether or not he would transmit the infection to the female rabbits. All rabbits were examined at monthly intervals.

TABLE II *Treponema paraluis-cuniculi infection in male laboratory rabbits infected intradermally with 2 × 10^7 treponemes on the shaved back*

	Rabbit No									
Response to infection	*104*	*23*	*15*	*14*	*939*	*940*	*21*	*22*	*17*	*16*
Latent period of infection (days)*	6	6	6	6	6	20	55	8	10	6
Maximum size (diameter) of indurated lesion (mm)	6	9	18	6	6	±§	5	8	7	7
Time after inoculation when lesion most strongly indurated (days)	14	41	84	14	20	41**	136	10	55	10
Time after inoculation when lesions fully regressed (days)	55	>365†	>178‡	>365†	218	70	>365†	70	218	70
RPR test at 84 days after inoculation	+	±	+ +	+	+ +	+	±	+	+	±
TPHA at 84 days after inoculation (titre)	1/640	1/1280	>1/5120	1/1280	>1/5120	1/640	1/640	1/1280	1/1280	1/640

* Time between inoculation and first appearance of lesion

** Time of maximum zone of erythema (not induration) as lesion was only slightly indicated

† Experiment terminated at 365 days: lesions still present

‡ Rabbit destroyed at 178 days because of intercurrent ear infection

§ Very slight induration with a maximum zone of erythema of 18 mm diameter

+ + Very strongly positive; + strongly positive; ± weakly positive

The female rabbits developed genital lesions containing large numbers of treponemes about five months later (table III). The lesions developed as generalised inflammation and oedema of the labia, which later became crusted. It was not possible to quantify them. In all four rabbits lesions were transient even though treponemes could be readily isolated from the labial epidermis and mucosa by gentle scraping (table III), indicating that a rabbit may be infected and infectious without showing obvious lesions. Six months after cohabitation with the infected male all four female rabbits had developed specific antitreponemal antibodies, as shown by a positive TPHA titre, and three had also developed non-specific antibodies, as detected by the RPR test. One of the female rabbits (No 91249) may have been spontaneously cured by 10 months, when no lesions were seen and treponemes were not found in scrapings. This is uncertain however as the observation was terminated at that time.

TABLE III *Treponema paraluis-cuniculi infection in four female laboratory rabbits after cohabitation with an infected male rabbit*

Response to infection	*Rabbit No* 658	91326	91249	91271
Latent period of infection (months)*	3	6	5	5
Treponemes present in genital lesions or labial scrapings taken at:				
6 months†	+	+	+	+
7 months	+	+‡	+	+
8 months	+	+	+	+‡
9 months	+‡	+	+	+‡
10 months	+	+	−‡	+
RPR test result at:				
6 months	±	−	±	±
8 months	+	−	+	+
10 months	+ +	−	+ +	±
TPHA titre at:				
6 months	1/5120	1/640	>1/5120	1/320
8 months	1/5120	1/1280	1/1280	1/320
10 months	1/5120	1/1280	1/640	1/640

* Period between cohabitation with male rabbit and the development of genital lesions containing treponemes

† Infected male rabbit 82673 removed at 6 months and normal male rabbit 83324 placed in the pen to detect female-to-male venereal transmission

‡ No macroscopically visible genital lesions present

+ + Very strongly positive; + strongly positive; ± weakly positive RPR test: − negative

Female-to-male transmission

The four venereally infected female rabbits (Nos 658, 91326, 91249, and 91271), with treponeme-containing genital lesions after six months' cohabitation with the originally infected male rabbit (table III), were placed in a pen with one normal male rabbit (No 83324). This male rabbit was also examined monthly. He developed treponeme-containing genital lesions after five months' cohabitation with the four infected female rabbits, when the TPHA gave a positive result at a titre of 1/320 but the result of the RPR test was negative. Observations were terminated at that time as proof of venereal transfer had been obtained.

Reproductive abilities

Male rabbits, infected intratesticularly with *T paraluis-cuniculi,* were able to sire normal healthy litters. Female rabbits, infected venereally, were also able to produce apparently healthy offspring. Two such kittens of infected mothers were examined serologically and found to have negative results to the RPR test even though the mother had treponemal antibodies.

Discussion

For many years rabbits have been used for research into human syphilis, primarily because they were susceptible to the human pathogen *T pallidum* and the disease closely resembled primary syphilis in man. It was not until 1912 however that Ross[7] suggested that the anogenital lesions seen in some rabbits may represent a spirochaetal infection. Bayon[8] examined these lesions and found spirochaetes 8-12 μ in length, with 5-8 spirals, which were indistinguishable from *T pallidum.* He concluded that this previously unrecognised spontaneous spirochaetal disease of rabbits may be a source of error in experiments on human syphilis in the rabbit. Several papers have reviewed the history of work on this disease[4 9 10] and several give extensive clinical and histological details of the disease after natural and artificial infection.[4 11-14]

The disease is apparently widespread in European wild and laboratory rabbits, North American laboratory rabbits, and European hares,[1] although there are no reports of it being present or absent in other parts of the world. On the basis of our serological survey of Victorian rabbits we tentatively conclude that the disease is absent from Australia. The reason for this must presumably be that the rabbits which became successfully established as feral populations were free of the disease, either because the introduced rabbits were carefully selected or fortuitously. It is unlikely that a disease which does not cause rabbits to become systemically ill would have resulted in their keepers killing them off on the long sea voyage to Australia. There are other instances of parasites of European wild or domestic rabbits which either did not arrive or failed to become established in Australia, for example, *Spilopsyllus cuniculi,* the European rabbit flea,[15] and *Encephalitozoon cuniculi.*[16] On the basis of a study of sera from 20 Victorian hares which gave negative serological results treponemal disease is probably absent from this population also, although 27% of European hares had positive serological results for syphilis.[1]

The reported incidence of rabbit venereal spirochaetosis in naturally infected populations varies considerably. McLeod and Turner[14] found only six rabbits out of 1800 with treponeme-containing genital lesions in a survey of North American laboratory rabbits. Fried and Orlov[17] reported on two epidemics of rabbit venereal spirochaetosis in Moscow in 1927 and 1928. Only 0·7% of rabbits less than 8 months old were infected whereas 46·4% of rabbits older than 1 year were infected, suggesting its venereal mode of transmission. Artz and Kerl[18] found an incidence of 26·9% in adult European rabbits and Noguchi[11] reported incidences of 10% and 30% in North American laboratory rabbits. Adams *et al*[13] reported the disease to be endemic in British wild and hutch rabbits and found 14 of 228 laboratory rabbits with treponeme-containing genital lesions. He further added that "reputable observers" estimated that 10-40% of wild rabbits were affected.

Other surveys have been based on serological methods using tests designed for the diagnosis of human syphilis. A number of these reports are recorded by Smith and Pesetsky[9] using non-specific tests such as the Wassermann reaction and the Venereal Disease Research Laboratory (VDRL) test, both of which detect antibody to cardiolipin, as does the RPR test used in our study.

There is considerable difference of opinion on whether or not the disease gives rise to positive results to cardiolipin tests; some argue that it does[4 19 20] while others have found their results to be uniformly negative.[11 12] Our controlled experiments with venereally and experimentally infected rabbits may have settled this controversy. While most rabbits (17/19) did develop positive results to the RPR test, two did not (rabbits Nos 91326 and 83324), although treponeme-containing genital lesions were present. The result is obviously variable. With the more specific (and probably more sensitive) TPHA however all rabbits with lesions gave positive results (19/19). This is a fairly recent test and has not previously been used in rabbit venereal spirochaetosis. Other specific antitreponemal serological tests however have been used. Pannu *et al*[20] studied 30 normal rabbits, 10 of which had a positive VDRL test result, and found two had weakly reactive results to the fluorescent treponemal antibody absorbed (FTA-ABS) test, although in one case the result subsequently became non-reactive. Twenty-nine of 30 rabbits gave negative results to the *Treponema pallidum* immobilisation (TPI) test and the result on the other rabbit was not stated in the paper. There was no clinical or bacteriological evidence however that these rabbits were infected. Small and Newman[4] detected positive FTA-ABS test results in known infected rabbits with peak titres at the time of appearance of cutaneous lesions. They also detected positive RPR test results in all infected rabbits in contrast to our own observations. Significant titres (in the RPR and FTA-ABS tests) were present six months after inoculation at the end of their experiment.

In retrospect it appears that it may have been wiser to screen the wild Victorian rabbit sera with the TPHA rather than with the RPR test as the former gave uniformly positive results in infection. The RPR test was used however because it is generally considered to be the appropriate screening test in serological surveys of syphilis. Nevertheless, it is unlikely that our conclusions on the absence of this disease in wild Victorian rabbits would be altered by retesting all sera with the TPHA. The eight RPR-positive (or weakly positive) sera were all TPHA-negative and these are the sera most likely to have been from *T paraluis-cuniculi* infected rabbits.

Our demonstration of venereal transmission of *T paraluis-cuniculi* between rabbits was not new; others have previously done so.[11-13] However, our experiments did show that Australian laboratory rabbits were readily susceptible to this bacterium and that the absence of infection in the wild population, including rabbits known to be partly domestic in origin, is probably not due to innate rabbit resistance to the disease but to a failure of the pathogen to be introduced.

Our observation that *T paraluis-cuniculi* could be readily isolated from labial scrapings in infected female rabbits, even in the absence of microscopic lesions, has also been reported.[13] While our rabbits developed genital lesions about five months after cohabitation with infected mates, Noguchi[11] reported that a male rabbit developed lesions after three months in a similar situation. These time periods are not significantly different.

Two of the offspring of an infected, serologically positive female rabbit were themselves RPR-negative and apparently normal in every respect. This suggests that the infection did not become systemic and that a bacteraemia was not established, supporting earlier observations.[12 13] In general, maternal rabbit antibodies of any immunoglobulin class will pass into the fetal circulation,[21] so our failure to detect antibodies reacting in the RPR test in these newborn rabbits is difficult to understand.

We wish to thank Mr Ian McLean and Mr Chris Roberts for their excellent technical assistance.

This work was supported by grants from the National Health and Medical Research Council, the

Utah Foundation, the Danks Trust, the estate of the late George Adams, the Ian Potter Foundation, and Monash University, from whom funds are gratefully acknowledged. The collection of sera was supported by the Wool Research Trust Fund.

References

1. Horvath I, Kemenes F, Molnar L, Szeky A, Racz I. Experimental syphilis and serological examination for treponematosis in hares. *Infect Immun* 1980;**27:**231-4.
2. Edmonds JW. Immunoglobulin allotypes of wild rabbits *Oryctolagus cuniculus* (L) in south-eastern Australia. Thesis, Melbourne, Australia: Monash University, 1977.
3. Turner TB, Hollander DH. Biology of the treponematoses. *World Health Organization Monograph Series No. 35,* Geneva: WHO, 1957.
4. Small JD, Newman B. Venereal spirochetosis of rabbits (rabbit syphilis) due to *Treponema cuniculi;* a clinical, serological and histopathological study. *Lab Anim Sci* 1972;**22:**77-89.
5. Shepherd RC, Nolan IF, Edmonds JW. A review of methods to capture live wild rabbits in Victoria. *Journal of Wildlife Management* 1978;**42:**179-84.
6. Graves S, Sandok P, Johnson R, Jenkin H. Retention of motility and virulence of *Treponema pallidum* (Nichols strain) in vitro. *Infect Immun* 1975;**12:**1116-20.
7. Ross EH. An intracellular parasite developing into spirochaetes. *Br Med J* 1912;ii:1651-4.
8. Bayon H. A new species of treponema found in the genital sores of rabbits. *Br Med J* 1913;ii:1159.
9. Smith JL, Pesetsky BR. The current status of *Treponema cuniculi;* review of the literature. *Br J Vener Dis* 1967;**43:**117-27.
10. Smith JL. In: *Spirochaetes in Late Seronegative Syphilis, Penicillin Notwithstanding.* Springfield, Illinois, USA: Charles C Thomas, 1969:156-75.
11. Noguchi H. Venereal spirochaetosis in American rabbits. *J Exp Med* 1922;**35:**391-407.
12. Warthin AS, Buffington E, Wanstrom RC. A study of rabbit spirochetosis. *J Infect Dis* 1923;**32:**315-32.
13. Adams DK, Cappell DF, McCluskie JA. Cutaneous spirochaetosis due to *Treponema cuniculi* in British rabbits. *Journal of Pathology and Bacteriology* 1928;**31:**157-61.
14. McLeod C, Turner TB. Studies on the biologic relationship between the causative agent of syphilis, yaws and venereal spirochaetosis of rabbits. I Observations on *Treponema cuniculi* infections in rabbits. *Am J Syphilis* 1946;**30:**442-54.
15. Sobey WR, Conolly D. Myxomatosis: the introduction of the European rabbit flea (*Spilopsyllus cuniculi* Dale) into the wild rabbit populations in Australia. *J Hyg Camb* 1971;**69:**331-46.
16. Cox JC, Pye D, Edmonds JW, Shepherd RC. An investigation of *Encephalitozoon cuniculi* in the wild rabbit *Oryctolagus cuniculus* in Victoria. *J Hyg Camb* 1980;**84:**295-300.
17. Fried SM, Orlov SS. Spontaneous spirochaetosis and experimental syphilis in rabbits. *Archives of Dermatology and Syphilology (Chicago)* 1932;**25:**893-905.
18. Arzt L, Kerl W. Weitere mitteilung über spirochätenbefunde bei kaninchen. *Wien Klin Wochenschr* 1914;**27:**1053-5.
19. Clark JW. Serological tests for syphilis in healthy rabbits. *Br J Vener Dis* 1970;**46:**191-7.
20. Pannu JS, Rosenberg MA, Israel CN, Smith JL. Incidence of reactive VDRL tests in the normal rabbit. In: Smith JL, ed. *Spirochaetes in Late Seronegative Syphilis, Penicillin Notwithstanding.* Springfield, Illinois, USA: Charles C Thomas, 1969:176-8.
21. Beer AE. Billingham RE. *The Immunology of Mammalian Reproduction.* Eaglewood Cliffs, New Jersey, USA: Prentice Hall Inc, 1976.

BOOKS OF MAJOR INTEREST

Recent Advances in Sexually Transmitted Diseases — 2
Edited by *J. R. W. Harris*
1980 296 pages illustrated hardback £18.00
A world-wide team of experts review 22 topics of current international interest and importance in this field. Deals with a spectrum of subjects covering clinical, research, laboratory, epidemiological and control aspects of sexually transmitted diseases.

Infectious Diseases:
Epidemiology and Clinical Practice
A. B. Christie
1980 Third edition 1066 pages 34 full colour + 16 b/w half-tone illustrations hardback £37.50
The new updated edition of a very popular book providing in depth coverage of infectious diseases for both reading and reference. '. . .a work to prize.' *World Medicine*

Recent Advances in Clinical Virology — 2
Edited by *A. P. Waterson*
1980 208 pages illustrated hardback £16.00
Reviews of the previous volume include:
'. . .excellent review articles covering some of the most fascinating problems in modern virology. . .' *The Practitioner*

Tropical Venereology
O. P. Arya, A. C. Osoba and *F. J. Bennett*
1980 248 pages illustrated paperback £8.00
'. . .excellent. . .practical. . .up to date. . . valuable. . .' *Medicine Digest*

Churchill Livingstone
Robert Stevenson House, 1-3 Baxter's Place, Leith Walk, Edinburgh EH1 3AF U.K.

M.S.S.V.D.

The Medical Society for the Study of Venereal Diseases

11 Chandos Street, Cavendish Square, London W1M 0EB
President: Dr. C. B. S. Schofield

The object of the Society is to bring together those interested or engaged in the diagnosis, treatment, and public health aspects of venereal diseases. The Society meets regularly in London for the reading of papers, discussions, and presentation of cases. An out-of-London meeting is held in the Spring.

Annual subscription for membership of the Society is £12 or $22.00 for all members.

Applications for membership are very welcome and forms can be obtained from the Honorary Secretary, Dr. J. Barrow, Lydia Department, St. Thomas' Hospital, SE1 7EH.

Programme 1980-81

Friday November 28th	The T.P.I. test	Dr. A. E. Wilkinson Dr. J. B. Selkon Professor J. Thivolet Dr. J. L. Fluker
Friday 30th January, 1981	Intestinal Parasites	Professor Lewis M. Drusin Dr. A. McMillan Dr. J. W. Ackers
Friday 27th March, 1981	Vulval Disease	Professor Charles Douglas
Friday 24th April, 1981	Presidential Address Higher Medical Training in Venereology	

THEY CALL IT CRABS

You may know it as Phthirus pubis

Whatever you call them, pubic lice are a problem, because any measure you adopt to eradicate them must take care of both adult lice and nits, while being cosmetically acceptable. Quellada Shampoo fills the bill particularly well. Its active constituent, gamma benzene hexachloride, is rapidly lethal to crab lice, while the foaming shampoo base ensures that Quellada penetrates through to the nits. So a single application of Quellada kills lice *and* nits. Quellada Shampoo is easy and pleasant to use, because it doesn't smell or irritate the skin.

Quellada PC Shampoo

gamma benzene hexachloride 1%

kills pubic lice and nits in 4 minutes.

Presentation
Gamma benzene hexachloride 1% in a specially formulated foaming shampoo base.

Uses
Antiparasitic and ovacidal. For use on hairy areas of the body in the treatment of lice infestations, e.g. head lice, body lice, pubic lice.

Dosage and administration
Apply to wet hair, rub vigorously for four minutes, rinse and comb with fine-tooth comb.

Contra-indications, Warnings etc.
Avoid contact with the eyes. Should lather accidentally get into eyes, flush immediately with water. Do not use more than twice in any one infestation nor in any one week. Do not use on broken or infected skin.

Packaging and price
Bottles of 100 and 500ml. Basic NHS cost 60p for 100ml.

Product Licence No.
0036/5001.

Further information is available on request.

Stafford-Miller Limited,
Professional Relations Division,
Hatfield, Herts. AL10 0NZ.

Br J Vener Dis 1980;**56**:387-9

Susceptibility of rabbits venereally infected with Treponema paraluis-cuniculi to superinfections with Treponema pallidum

S GRAVES

From the Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran, Victoria, Australia

SUMMARY Three female rabbits, venereally infected with *Treponema paraluis-cuniculi* between five and eight months previously, had treponeme-containing genital lesions and positive results to serological tests for syphilis. These rabbits and four normal female rabbits were challenged with *Treponema pallidum* intradermally on the shaved back with triplicate doses of 10^4, 10^3, 10^2, and 10 treponemes (all at different sites). Significantly fewer syphilitic lesions developed in the rabbits previously infected with *T paraluis-cuniculi* (4/36) compared with the control rabbits (37/48), and the mean size of the lesions (diameter of induration) was significantly less (4 mm compared with 10 mm). It appears that previous venereal infection of female rabbits with *T paraluis-cuniculi* induced a level of cross-immunity against infection with *T pallidum*. Protection was however not complete.

Introduction

Treponema paraluis-cuniculi and *Treponema pallidum,* which cause rabbit venereal spirochaetosis and human syphilis respectively, are closely related morphologically[1] and antigenically.[2] Previous studies[2-4] have shown an appreciable level of immunity to *T pallidum* in rabbits inoculated intratesticularly with *T paraluis-cuniculi* between six and eight months previously.

In the experiment now reported.venereally infected female rabbits (the natural route of infection with *T paraluis-cuniculi*) were challenged with low serial 10-fold doses of *T pallidum* (10^4, 10^3, 10^2, and 10) intradermally on the shaved back, and the degree of syphilitic infection was measured by the percentage of inoculation sites at which lesions developed and their size. A comparison was made with normal female rabbits challenged with *T pallidum* to ascertain the degree of protection against *T pallidum* offered by the previous venereal infection with *T paraluis-cuniculi.*

Address for reprints: Dr S Graves, Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran, Victoria 3181, Australia

Received for publication 12 March 1980

Materials and methods

INFECTION WITH *T PARALUIS-CUNICULI*

A male rabbit (rabbit No 82673) was experimentally infected with *T paraluis-cuniculi* and placed in a pen with four normal female rabbits, as described.[5] The four female rabbits became venereally infected, as shown by the development of treponeme-containing lesions and seroconversion about five months after cohabitation with the infected male rabbit.[5] Six months afterwards, when lesions were still present in three of the four female rabbits, they were challenged with *T pallidum.*

CHALLENGE WITH *T PALLIDUM* (NICHOLS STRAIN)

T pallidum was obtained from the testes of a rabbit inoculated intratesticularly with 5×10^7 *T pallidum* about 10 days previously. Details of procedure and elution medium used were as published.[6] The same anaerobic medium was used to dilute the *T pallidum* to 10^5/ml and thence in serial 10-fold dilutions to 10^2/ml (under anaerobic conditions).

The four control and four *T paraluis-cuniculi*-infected rabbits had their backs shaved and marked into a 3 × 4 grid, each of the 12 squares being approximately 15 mm in length. Into each square 0·1 ml of *T pallidum* suspension was inoculated intradermally, so that each rabbit received triplicate inoculations of 10^4, 10^3, 10^2, and 10 *T pallidum.*

The rabbits were examined twice a week for five weeks and the development of indurated syphilitic lesions on the backs was recorded. The rabbits' backs were kept shaved during this period.

SEROLOGICAL MONITORING

The rapid plasma reagin (RPR) test was carried out according to the manufacturer's instructions (Commonwealth Serum Laboratories, Melbourne, Australia). Results were recorded as strongly positive, weakly positive, or negative. The *Treponema pallidum* haemagglutination assay (TPHA) was performed using the kit produced by Fujizoki Pharmaceutical Company Ltd. The manufacturer's instructions were followed. Only tires greater than 1/80 were considered positive and therefore indicative of past or current treponemal infection. Known positive and negative control sera were included in each test series. Previous studies have shown that results to these two tests become positive in venereal rabbit spirochaetosis.[5]

Results

One of the *T paraluis-cuniculi*-infected rabbits was excluded from the experimental analysis because it had been badly attacked by the male rabbit and had numerous mating bites and infected claw injuries on its back, making detection of classical indurated *T pallidum* lesions at the site of inoculation very difficult.

As shown in the table, the rabbits which had previously been infected venereally with *T paraluis-cuniculi* had developed some degree of protection against challenge with *T pallidum,* as shown by the smaller number of lesions which developed at the inoculation sites and their smaller size, even though the protection was not complete.

After venereal infection of the three female rabbits with *T paraluis-cuniculi* they developed positive results to the RPR test and TPHA. The uninfected control rabbits had negative results. After challenge with *T pallidum* the test rabbits continued to have positive results and the results for the control rabbits became positive in both serological tests.

Discussion

Turner and McLeod[4] and Turner *et al*[3] carried out studies on the susceptibility of rabbits experimentally infected intratesticularly with *T paraluis-cuniculi* to challenge with *T pallidum* (Nichols strain and strain CJ). They found considerable, but not complete, protection. As they comment, their experiments were a severe test of the immune system of the *T paraluis-cuniculi*-infected rabbits, as their intradermal challenge inocula were approximately 2×10^6 *T pallidum.* Nevertheless some protection was evident by the absence, or slow development, or smaller size of the lesions after challenge with *T pallidum.* Their results were sufficiently impressive for them to write, "While no (syphilis) vaccine is in sight, the problem (human syphilis) is sufficiently important to warrant the exploration of all avenues of approach, including the potentialities of *T cuniculi* as an immunising agent."

Subsequent work, reported by Turner and Hollander[2] involved inoculating rabbits intratesticularly with *T paraluis-cuniculi* (strain A) and eight months thereafter challenging with a low dose (500 treponemes) of *T pallidum* (Nichols) intradermally on the shaved back. As in the earlier experiments partial protection was obtained.

The purpose of the experiment reported in this paper was to determine whether or not rabbits naturally infected by the venereal route (as distinct from experimental intratesticular inoculation) with *T paraluis-cuniculi* were resistant to low challenge doses of *T pallidum* (Nichols) given intradermally. From the results it appears that significant protection did occur. Low challenges (10 and 10^2) of *T pallidum* failed to cause any observable lesions in the

TABLE *Development of syphilitic lesions in normal rabbits and in rabbits previously infected with Treponema paraluis-cuniculi on intradermal (id) challenge with four different doses of Treponema pallidum (Nichols strain)*

	*Normal female rabbits**		*T paraluis-cuniculi-infected female rabbits†*	
T pallidum challenge doses (id)	*No of lesions/sites inoculated*	*Diameter of lesions (mm) (mean ± SD)*	*No of lesions/sites inoculated*	*Diameter of lesions (mm) (mean ± SD)*
10^4	10/12	11·0±6·9	3/9	4·0±3·6
10^3	10/12	11·1±3·5	1/9	4·0
10^2	11/12	7·8±5·7	0/9	NL
10	6/12	8·3±3·6	0/9	NL
Total	37/48		4/36	

*Four rabbits
†Three rabbits
NL = no lesion

immunised rabbits and higher challenges (10^3 and 10^4) caused significantly fewer and significantly smaller lesions in the immunised rabbits (table).

It appears that *T paraluis-cuniculi* does offer some protection to rabbits against low challenge doses of *T pallidum*. Would it do the same for humans, and could it be a potential vaccine against syphilis? Further studies, in both rabbits and humans, are required to clarify this important question.

I wish to thank Mr Ian McLean for his excellent technical assistance and Mr John Edmonds for his valuable advice and assistance. The kind permission of the Director of the Keith Turnbull Research Institute (Mr B Woodfield) for use of the rabbit-pens is acknowledged.

This work was supported by grants from the National Health and Medical Research Council, the Utah Foundation, the Danks Trust, the estate of the late George Adams, the Ian Potter Foundation, and Monash University, from whom funds are gratefully acknowledged.

References

1. Hougen KH, Birch-Andersen A, Jensen HS. Electron microscopy of *Treponema cuniculi*. *Acta Pathol Microbiol Scand (B)* 1973;**81**:15-26.
2. Turner TB, Hollander DH. Biology of the treponematoses. *World Health Organisation Monograph series No. 35*, Geneva: WHO, 1957.
3. Turner TB, McLeod CP, Updyke EL. Cross-immunity in experimental syphilis, yaws and venereal spirochetosis of rabbits. *Amer J Hyg* 1947;**46**:287-95.
4. Turner TB, McLeod CP. Cross-immunity in experimental syphilis, yaws and venereal spirochetosis of rabbits. *Trans Assoc Am Physicians* 1942;**57**:265-6.
5. Graves SR, Edmonds J, Shepherd RC. Lack of serological evidence for venereal spirochetosis in wild Victorian rabbits and the susceptibility of laboratory rabbits to *Treponema paraluis-cuniculi*. *Br J Vener Dis* 1980;**56**:381-6.
6. Graves SR, Sandok PL, Jenkin HM, Johnson RC. Retention of motility and virulence of *Treponema pallidum* (Nichols strain) in vitro. *Infect Immun* 1975;**12** (5):1116-20.

Br J Vener Dis 1980;**56**:390-3

Assessment of transport and isolation methods for gonococci

E TAYLOR AND I PHILLIPS
From the Department of Microbiology, St Thomas's Hospital Medical School, London

SUMMARY Urethral discharge from men was diluted to give heavy and light inocula and cultured on seven different solid culture media, including two transport/isolation media, or held in three types of semi-solid transport medium for varying periods and then cultured. The amount of growth was quantitated and the performance of the different systems compared. Fresh non-selective media were best, with up to two failures in 254 cultures on each medium. With selective media there were 9-23 failures with heavy inocula and 22-47 failures with dilute inocula. For Transgrow or the Jembec system incubation before holding at ambient temperature was better than holding followed by incubation. Transport media yielded good results if cultures were set up within six hours; only minor losses occurred after 24 hours.

Introduction

Many methods have been suggested for the transport of samples from patients suspected of having gonorrhoea and for the primary isolation of *Neisseria gonorrhoeae*. Various transport media, isolation media inoculated in the clinic, and more recently media for the combined transport and growth of the organism have all been used. We have carried out an assessment of some of these methods.

Materials and methods

URETHRAL SAMPLES

Samples of urethral discharge containing Gram-negative intracellular diplococci, which was sufficiently profuse, were collected both for routine diagnosis and for this study from men attending the department of genitourinary medicine at St Thomas's Hospital, London.

MEDIA

Seven preparations of solid culture media, three solid transport/isolation media, and three types of semi-solid transport medium were assessed.

The solid media (A-G) were either supplied ready-poured in divided plates by Difco Ltd or Oxoid Ltd or were prepared in St Thomas's Hospital (table I). The formulae of these media were as follows:

GC non-selective (A) (Difco)—proteose peptone No 3 15 g/l, corn starch 1 g/l, dipotassium phosphate 4 g/l, monopotassium phosphate 1 g/l, sodium chloride 5 g/l, agar 15 g/l, haemoglobin 10 g/l, dextrose 1 g/l, and supplement B 10 ml/l;

GC selective (B) (Difco)—as above, plus vancomycin 3 mg/l, colistin sulphomethate 7·5 mg/l, nystatin 12 500 units/l, and trimethoprim 5 mg/l;

Transgrow[1] (C) (Difco)—as GC selective, but with agar 20 g/l, supplement B 20 ml/l;

VCNT[2] (D, E, and F)—heart infusion agar (Difco 0044-01), saponin-lysed horse blood 10% (heated blood was used for the medium in Jembec plates), plus antibiotics as above; and

Lysed blood agar (G) (St Thomas's Hospital)—DST agar (Oxoid CM-261), saponin-lysed horse blood.

The three solid transport/isolation systems provided by Oxoid were:

Transgrow in bottles;

VCNT in Jembec plates[3]; and

Chocolate agar VCNT in Jembec plates.

The semi-solid transport media were:

Stuart's transport medium (Southern Group Laboratories)—sodium glycerophosphate 10 g/l, thioglycollic acid 1 g/l, calcium chloride 1 g/l, methylene blue 0·02 g/l, and agar (Becton Dickson) 2·2 g/l;

Stuart's transport medium (Oxoid R22)—sodium glycerophosphate 10 g/l, sodium thioglycollate 0·5 g/l, cysteine hydrochloride 0·5 g/l, calcium chloride 0·1 g/l, methylene blue 0·001 g/l, and agar No 1 (Oxoid L11) 10 g/l; and

Amies's transport medium (Oxoid 425)—sodium

Address for reprints: Professor I Phillips, Department of Microbiology, St Thomas's Hospital Medical School, London SE1 7EH

Received for publication 31 December 1980

TABLE I *Bacterial counts from small inocula on seven different media**

	Transgrow (C)	*GC selective (B)*	*VCNT (D)*	*VCNT (E)*	*VCNT (F)*	*Lysed-blood agar (G)*	*GC non-selective (A)*
Container	Bottle	Petri dish	Petri dish	Jembec	Petri dish	Petri dish	Petri dish
Poured by	Difco	Difco	Oxoid	Oxoid	St Thomas's	St Thomas's	Difco
No of observations	253	250	254	114	254	243	245
Mean No of colonies/plate	25	27	30	31	32	40	41

* In order of increasing efficiency

chloride 8 g/l, potassium chloride 0·2 g/l, calcium chloride 0·1 g/l, magnesium chloride 0·1 g/l, monopotassium phosphate 0·2 g/l, disodium phosphate 1·5 g/l, sodium thioglycollate 1·0 g/l, charcoal 10 g/l, and agar 3·6 g/l.

EXPERIMENT 1

The solid media for isolation of *N gonorrhoeae* were compared in the first experiment.

The sample of urethral pus was collected on a plain swab, which was broken off into 1 ml of nutrient broth containing 10% lysed horse blood, and brought to the laboratory within two hours. When the sample was received in the laboratory the swab was discarded after the liquid had been expressed from it. The broth and a 10^{-3} dilution of it were used in standard loopfuls of 0·007 ml to inoculate the various types of solid medium listed in table I in parallel. Cultures were then incubated overnight at 36°C in 10% CO_2, except for the Transgrow bottle, which already contained CO_2, and the Jembec plate, which included a tablet from which CO_2 was generated.

The growth from a heavy inoculum was roughly quantitated but the individual colonies that grew from light inocula were counted whenever possible. Total counts for light inocula of each sample were compared by paired t-tests provided at least one plate had more than 10 colonies in the inoculum and provided none had more than 200 colonies. An analysis of variance was also performed on the counts from light inocula. The various patterns of complete failure of isolation were enumerated for cultures from heavy and light inocula.

EXPERIMENT 2

The solid combined transport and culture media were compared in the second experiment.

Three solid media, Transgrow and VCNT with lysed or heated blood in Jembec plates, were inoculated with heavy inocula from lysed-blood broth as in the first experiment and then either left at room temperature for periods of up to two days before incubation or incubated overnight and then left for periods of up to four days before subculture.

EXPERIMENT 3

The semi-solid transport media were compared in the third experiment.

Six swabs were soaked in the heavy lysed-blood broth suspension of gonococci prepared for the first experiment and placed in pairs of each of the three types of semi-solid transport medium. One of each pair was held at 4°C and the other at room temperature. The swab was used to provide subcultures to warm lysed-blood agar at daily intervals for five days. It was shown in a preliminary experiment that repeated use of the same swab did not significantly deplete it of gonococci.

ORGANISMS

Gonococci were identified as in previous investigations by Gram-staining, the oxidase test, and specific immunofluorescence. Sugar fermentation tests were also performed for the few organisms that did not fluoresce.

Results

SOLID MEDIA

The results of *N gonorrhoeae* counts on the various media for 254 parallel cultures from dilute inocula in experiment 1 are shown in table I. Results of a further 70 cultures were excluded because the number of colonies growing from the dilute inoculum did not exceed 10 on any medium; those of a further six cultures were also excluded because counts were too high. The total number of observations on each medium varied because plates were occasionally contaminated or unavailable—the latter especially with medium E (table I). On the basis of paired t-tests[4] the two non-selective media, A (GC non-selective) and G (lysed-blood agar), did not differ from each other, but there was a significant difference between these two and the rest and between media C (Transgrow) and F (St Thomas's VCNT).

With low inocula 159 (63%) cultures grew the organism on all plates. Failures were commonest on selective media B (47), D (40), C (25), and F (22), were uncommon on lysed-blood agar (G) (2), and did

TABLE II *Viability of gonococci in three transport-culture systems*

Medium for transport/culture	Incubation	% Failure from cultures or subcultures after: 6 hrs	24 hrs	2 days	3 days	4 days	No of tests
Transgrow	Delayed	2·4	5·7	33·6	NT	NT	122
	Immediate	0	1·4	4·0	8·8	24·3	148
VCNT in Jembec plate	Delayed	2	26	86	NT	NT	50
	Immediate	0	2	16	70	92	60
Chocolate VCNT in Jembec plate	Delayed	0	14	68	NT	NT	50
	Immediate	0	4	16·7	26·2	81	81

NT = not tested

not occur with medium A. Results for medium E came from only 84 cultures, and a corrected failure rate for 254 cultures would be 24, making the medium similar to C and F.

When the larger inocula were considered in the same way 319 of 334 cultures gave assessable results. In 88% gonococci were isolated from all media. Failures were commonest on selective media B (23 failures), D (19), C (15), E (6, or 11 after correction), and F (9), and were very uncommon on the non-selective media A (2) and G (1).

TRANSPORT/CULTURE MEDIA

The results of tests for the viability of gonococci on the three solid transport culture media (experiment 2) either kept for up to two days before incubation (delayed) or first incubated (immediate) and then left at room temperature for up to four days before subculture are shown in table II. In the "delayed" series, in most instances incubation within six hours yielded gonococci but incubation after 24 hours resulted in a 5·7% loss of isolates from Transgrow in bottles, and 14% from chocolate agar, and 26% from lysed-blood agar in Jembec plates; after two days these figures were 33·6%, 68%, and 86% respectively.

We noted a marked seasonal variation in results for media held at room temperature before incubation. During the period January to March failure rates for Transgrow were 4% after six hours, 10% after 24 hours, and 54% after 48 hours. During April to June the respective figures were 2%, 4%, and 26%, and in the hot months of July and August (room temperature reaching 35·5°C) there were no losses among 22 cultures kept for up to 48 hours before incubation. For Jembec plates the figures were 2% after six hours, 26% after 24 hours, and 86% after 48 hours for January to March, and 0%, 14%, and 68% respectively for April to June.

In the "immediate" series, in which cultures were incubated overnight and then examined, sub-cultures within six hours all yielded viable gonococci. A delay of 24 hours before subculture resulted in some loss of viability, and this increased over the four days of the study. In the Transgrow system only a quarter of the cultures were not viable after four days at room temperature whereas in the other two systems most of the cultures had died by this stage.

SEMI-SOLID TRANSPORT MEDIA

The results for experiment 3 on semi-solid transport media are shown in table III. Overall, Amies's medium was best, and the modification of Stuart's medium containing more agar was worst. Storage at 4°C was more efficient for Stuart's media but not for Amies's medium. Even with the best system used, a quarter of the cultures were dead after two days and 60% after four days.

Discussion

The first experiment was an investigation of combined transport and culture media under good laboratory conditions. We showed, not surprisingly, that for cultures from men non-selective media are best. This superiority was demonstrated by our finding significantly higher mean colony counts on non-selective media than on any other antibiotic-containing media and higher isolation rates. With

TABLE III *Viability of gonococci in three semi-solid transport systems*

Transport medium	Temperature of storage	% Failure from cultures or subcultures after: 6 hrs	24 hrs	48 hrs	72 hrs	96 hrs	No of tests
Stuart's agar 1%	Room	0	79	97	100	100	58
(Oxoid R22)	4°C	0	41	95·5	100	100	22
Stuart's agar 0·22%	Room	0	41	77·3	95·5	100	22
(Southern Group)	4°C	0	4·5	36·4	63·6	91	22
Amies's	Room	0	5·2	24	36·2	60·3	58
(Oxoid)	4°C	0	13·7	32	41	59·1	22

heavy or light inocula there were only two or three complete failures on the non-selective media.

There was no clear difference for dilute inocula between the various selective media, the mean colony count ranging from 25 to 32. In terms of outright failure, Difco GC selective medium (B) and Oxoid VCNT (D) fared worst with 47 and 40 failures respectively from dilute inocula; Difco Transgrow (C), Oxoid VCNT with chocolate agar in Jembec plates (E), and St Thomas's Hospital VCNT (F) fared better and approximately equally with 25, 24 (a corrected figure), and 22 failures respectively. Results from heavy inocula were better, but failures—although less common—were seen more often with media B and D than with C, E, and F. It would seem that the use of dilute inocula did in fact constitute a more severe test of the media.

We feel that our overall results support our practice of using selective media for the isolation of *N gonorrhoeae,* even from men (from whom inocula are usually large), and that difficulties in quality control as applied to the commercial products probably accounted for the minor differences that we observed. Clearly, some kind of quality control of media for the isolation of gonococci is essential in the laboratory in which they are used. The most sensitive test in our experience is of their ability to support the growth of small inocula of *N gonorrhoeae* in a series of tests, but this is unfortunately impossible in most laboratories. Growth from the natural inoculum from urethral samples from men with positive results by Gram staining is a practicable alternative.

The second part of the study attempted to simulate delays in combined transport-isolation media reaching the laboratory, as for example by post, and the effect of incubation before or after such a delay. For all our systems, incubation before dispatch was clearly better. This is in contrast to Brown[5], who found that it made little difference with his modified Transgrow. The Transgrow system was better than the Jembec system with the media that we used; for example, two days after primary incubation only 4% of the Transgrow cultures were dead. We emphasise that, unlike Jephcott *et al,*[3] we did not use Transgrow in Jembec plates.

If cultures were held before incubation there was a striking loss of viability. Even in the Transgrow system one third of the cultures were dead by the second day. Jephcott *et al*[6] did not detect such deficiencies but studied Transgrow only under conditions of no delay. Chapel and his colleagues[7] held Transgrow inoculated with clinical samples at room temperature for 48 hours before incubation and found that about a quarter of the cultures giving positive results on immediate inoculation on Thayer-Martin medium failed on Transgrow, a result similar to ours. We conclude that although these transport-culture media performed well if incubated immediately and examined immediately, performance was not nearly so good under conditions that mimic more closely those actually obtaining in most clinics.

The final experiment compared three semi-solid transport media. All three were good, provided cultures were set up from them within six hours. By 24 hours failure was the rule with a modified Stuart's medium containing more agar, although in normal Stuart's and in Amies's media there were only 4-5% failures, depending on the temperature at which the transport media had been held. We are unable to explain our finding that storage at 4°C was better than at room temperature for Stuart's medium but worse for Amies's medium.

Our results draw attention to the problem of delay in setting up or incubating cultures for *N gonorrhoeae.* None of the transport methods fully compensates for delay but for the ones that we studied a delay of 24 hours had least effect on Transgrow incubated and then sent to the laboratory. Slightly inferior results were obtained with Jembec plates incubated and then sent to the laboratory, with Stuart's medium held at 4°C, and with Amies's medium held at room temperature.

We thank Dr C S Nicol and his staff for their participation in this study, which was made possible by a DHSS grant. We also thank Mr M Stoate and Dr S E Smith for their help with statistical analyses.

References

1. Martin JE, Lester A. Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis. HSMHA Health Reports* 1971;**86:**30-3.
2. Phillips I, Humphrey D, Middleton A, Nicol CS. Diagnosis of gonorrhoea by culture on a selective medium containing vancomycin, colistin, nystatin, and trimethoprim (VCNT): a comparison with Gram-staining and immunofluorescence. *Br J Vener Dis* 1972;**48:**287-92.
3. Jephcott AE, Bhattacharyya MN, Jackson DH. Improved transport and culture system for the rapid diagnosis of gonorrhoea. *Br J Vener Dis* 1976;**52:**250-2.
4. Duncan DB. Multiple range and multiple F tests. *Biometrics* 1955;**11:**1-42.
5. Brown JD. Modified transport and growth medium for the cultivation of *Neisseria gonorrhoeae. Br J Vener Dis* 1974;**50:**199-201.
6. Jephcott AE, Morton RS, Turner EB. Use of transport and culture medium combined with immunofluorescence for the diagnosis of gonorrhoea. *Lancet* 1974;ii:1311-3.
7. Chapel T, Smeltzer M, Printz O. An evaluation of commercially supplied Transgrow and Amies media for the detection of *Neisseria gonorrhoeae. Health Lab Sci* 1974;**11:**28-33.

Br J Vener Dis 1980;**56**:394-6

Gentamicin in the treatment of infections due to penicillinase-producing gonococci

N J TAN,* V S RAJAN,* ROGER PANG,* AND E H SNG†

*From the *Middle Road Hospital and the †Department of Pathology, Singapore General Hospital, Singapore*

SUMMARY Ninety-seven patients with infections due to penicillinase-producing *Neisseria gonorrhoeae* (PPNG) were treated with single doses of gentamicin 280 mg intramuscularly. The failure rate among those followed up was 3%. No significant side effects were recorded. Gentamicin is a valuable alternative in the treatment of infections due to PPNG strains.

Introduction

Gonorrhoea is a major public health problem and the sudden appearance of strains of gonococci that have acquired the ability to produce penicillinase, and so become totally resistant to penicillin and analogues of penicillin, has aggravated the situation. These strains were reported early in 1976,[1 2] and in the following years have established themselves in African and Asian countries.

The newer cephalosporins have been shown to be effective in the treatment of patients infected by penicillinase-producing strains of *Neisseria gonorrhoeae* (PPNG). Good results were reported with the use of cefuroxime[3] and HR 756.[4] These drugs are, as yet, generally unavailable and are likely to be expensive when introduced into the market. The aminoglycosides have already been shown to be efficacious in its treatment in the United States[5] and in Britain.[3] Rajan *et al*[6] obtained excellent results using kanamycin in a single dose. Strains of PPNG were reported to be susceptible in vitro to gentamicin,[5] and hence in vivo this aminoglycoside should be a useful addition. For this reason, a trial was conducted with gentamicin sulphate in the treatment of patients with gonorrhoea due to PPNG.

Patients and methods

Patients harbouring strains of PPNG seen between March and April 1979 were included in the study. Patients who had a history of allergy to gentamicin and other aminoglycosides and those with other sexually transmissible diseases were excluded from the trial. Most of the patients were prostitutes; the remainder consisted of patients who came for treatment and their sexual partners.

DIAGNOSIS AND TREATMENT

Infections in the prostitutes were detected during their routine fortnightly attendances, when cervical and rectal specimens were cultured on modified Thayer-Martin medium[7] for *N gonorrhoeae*, which was identified by colonial morphology, Gram staining, and the oxidase reaction. Penicillinase production was detected by screening positive gonococcal isolates by a modification of the filter paper iodometric technique of Odugbemi *et al.*[8] Strains giving positive results were confirmed by the rapid iodometric method.[9]

Urethral swabs were taken from all male patients for Gram staining and culture for *N gonorrhoeae*. When smears showed Gram-negative intracellular diplococci, the patient was treated provisionally as having gonorrhoea and was given our usual regimen, procaine penicillin 4·5 megaunits intramuscularly and probenecid 1 g orally. Infection by penicillinase-producing gonococci was diagnosed when on follow-up gonococci were still present and the pretreatment culture gave a growth of *N gonorrhoeae* which was shown to produce penicillinase.

In female patients and prostitutes treatment was begun only after the culture results were available.

Those patients infected with PPNG strains were treated with a dose of gentamicin 280 mg intramuscularly and seen three days later, when urethral cultures in men and endocervical and rectal cultures in women were repeated. Thereafter cultures were repeated at one week and two weeks after treatment. In addition, the serum creatinine concentrations were determined before treatment and repeated on the seventh day after treatment. A patient was considered cured when all the culture results were

Address for reprints: Dr V S Rajan, Middle Road Hospital, Singapore 7, Republic of Singapore

Received for publication 12 March 1980

negative. Treatment failure was considered to have occurred when one or more of the post-treatment cultures yielded PPNG and reinfection had been ruled out.

SENSITIVITY TESTS
The minimum inhibitory concentration (MIC) of gentamicin was determined by the agar-plate dilution method. Two-fold concentrations of gentamicin from 0·5 μg/ml to 32 μg/ml were distributed into GC agar base (Difco) supplemented with haemoglobin and IsoVitalex. The test organisms were grown on chocolate agar and then suspended in Mueller-Hinton broth. The suspension turbidity was adjusted to correspond to 0·5 McFarland standard barium sulphate. The suspension was further diluted 1/100, and approximately 10 μl of each suspension was inoculated on to the antibiotic-containing medium by a multipoint inoculator. The plates were incubated in CO_2 tins at 35°C overnight. The MIC was determined as the lowest concentration of antibiotic that permitted the growth of no more than one colony.

Results

Ninety-seven patients with infections due to PPNG were treated with gentamicin (table I). Of these 83 were prostitutes; of the remaining 14, 11 patients were male and three female. All the prostitutes attended for follow-up culture examinations after treatment. One male patient failed to reattend.

Of the 96 patients examined bacteriologically after treatment with gentamicin, three (all prostitutes) had persistent infections. They were treated with kanamycin 2 g intramuscularly and responded well. In the remaining 93 patients their infections were cured, giving a cure rate of 97%.

There were no obvious side effects. The serum creatinine values remained within the normal range. The patients tolerated the injections very well with little discomfort. Only one patient complained of moderately severe giddiness half an hour after the injection. This was transient and lasted for about two hours.

TABLE I *Results of treatment of infections due to strains of PPNG with gentamicin sulphate 280 mg intramuscularly*

			Total	
Results	*Prostitutes*	*Others*	*No*	*%*
Treatment failure	3	0	3	3
Cured	80	13	93	96
Defaulted	0	1	1	1
Total	83	14	97	100

SENSITIVITY TESTS
The sensitivity of the penicillinase-producing gonococci to gentamicin was tested in 72 of the patients (table II). All strains were susceptible to 8 μg/ml or less of the antibiotic. Strains from the three treatment failures were sensitive to 8 μg/ml gentamicin.

Discussion

Soon after their emergence early in 1976, penicillinase-producing gonococci gained a foothold in Africa and in the Far East. Although these strains are susceptible to some of the aminoglycoside antibiotics, there is still a need to look for alternatives.

In this study a cure rate of 97% was obtained with gentamicin when used in a single dose of 280 mg. The PPNG strains recovered from the three patients who failed to respond to treatment were sensitive to 8 μg/ml gentamicin. This concentration is easily obtained in tissue with the dosage used. Persistence of the infection in these three patients could thus have been due to reinfection. Taking this into consideration the actual cure rate may in fact have been higher.

TABLE II *Sensitivity of N gonorrhoeae to gentamicin*

MIC (μg/ml)	*No of strains*
2	1
4	14
8	57

None of the patients treated showed evidence of ototoxicity or renal toxicity. Published evidence[10] has suggested that these side effects should not occur when only one or two doses are given. Gentamicin in similar dosages has been used to treat uncomplicated gonococcal infection.[11] [12] None of these workers observed any serious side effects.

Gentamicin fulfils many of the criteria of a useful agent for the treatment of infections due to penicillinase-producing gonococci. It is effective, easily administered, and requires only a single dose, making it a valuable alternative for the treatment of PPNG infections. Because of the good treatment response obtained in this study, its use should be discouraged in the treatment of infections due to non-penicillinase-producing strains of *N gonorrhoeae*. Its indiscriminate widespread use may encourage the emergence of resistant strains, thus diminishing its usefulness as a valuable alternative antibiotic for the more difficult penicillinase-producing strains of *N gonorrhoeae*.

The authors wish to thank Schering Corporation, USA, for supplies of gentamicin.

References

1. Ashford WA, Golash KG, Hemming VG. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;ii:657-8.
2. Phillips I. Beta-lactamase-producing penicillin-resistant gonococcus. *Lancet* 1976;ii:656-7.
3. Arya OP, Rees E, Percival A, Alergant CD, Annels EA, Turner GC. Epidemiology and treatment of gonorrhoea caused by penicillinase-producing strains in Liverpool. *Br J Vener Dis* 1978;**54**:28-35.
4. Rajan, VS, Sng EH, Pang R, Tan NJ, Thirumoorthy T, Yeo KL. HR 756: a new cephalosporin in the treatment of gonorrhoea caused by ordinary and penicillinase-producing strains of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1980;**56**:255-8.
5. Center for Disease Control. *Morbidity and Mortality Weekly Report* 1976;**25**:261.
6. Rajan VS, Pang R, Tan NJ, Sng EH. Kanamycin in the treatment of penicillinase-producing gonococcal infections. *Asian J Infect Dis* 1979;**3**:37-9.
7. Martin JE, Armstrong JH, Smith PB. A new system for cultivation of *Neisseria gonorrhoeae*. *Appl Microbiol* 1974;**27**:802-5.
8. Odugbemi TO, Hafiz S, McEntegart MG. Penicillinase-producing *Neisseria gonorrhoeae:* detection by starch paper technique. *Br Med J* 1977;ii:500.
9. Catlin BW. Iodometric detection of *Haemophilus influenzae* beta-lactamase: rapid presumptive test for ampicillin resistance. *Antimicrob Agents Chemother* 1975;**7**:265-9.
10. Felarca AB, Laqui EM, Ibarra LM. Therapeutic dose response and tolerance studies on gentamicin in gonococcal urethritis. *J Infect Dis* 1971;**124** suppl;287.
11. Hantschke D, Strauss P, Linzenmeier G, Gahlen D, Heller W. Treatment of gonorrhoea with single injection of gentamicin. *Br J Vener Dis* 1973;**49**:62-4.
12. Morrison GD, Reeves DS. Gentamicin in the treatment of gonococcal urethritis. A microbiological, pharmacological and clinical study. *Br J Vener Dis* 1973;**49**:513-6.

Br J Vener Dis 1980;**56**:397-9

Rectal gonorrhoea in male homosexuals

Presentation and therapy

J L FLUKER,* P DEHEROGODA,* D J PLATT,† AND A GERKEN†

*From the Departments of *Genitourinary Medicine and †Medical Microbiology, Charing Cross Hospital, London*

SUMMARY In a review of rectal gonorrhoea 73 episodes were studied in 65 homosexual men. The presenting signs and symptoms were carefully noted. Treatment with a single injection of spectinomycin hydrochloride 2 g resulted in a cure rate of 94·5%. The relatively high treatment failure rate associated with rectal gonorrhoea may possibly be due to microbial mechanisms.

Introduction

The continuing high prevalence of venereal and, to a lesser extent, other sexually transmissible diseases in the male homosexual community emphasises the need to review the clinical presentation, microbiology, and treatment of rectal gonorrhoea regularly. The department of genitourinary medicine at the Charing Cross Hospital, London, is attended by an exceptionally large proportion of homosexual patients.

Of 15 236 cases of male gonorrhoea diagnosed during the years 1962-71, 3430 (22·5%) were homosexually acquired and comprised 1867 (54·4%) rectal and 1563 (45·6%) urethral infections. Since 1968 the proportion of homosexual cases has fluctuated between 26% and 31% of the gonococcal infections diagnosed in men each year.[1] Of the 1867 patients with rectal infections 988 (53%) had no symptoms whatsoever and attended either for routine examination or because their sexual contacts had so advised.

The commonest complaints were a slight irritation (in 839, 45%) and a mucoid discharge (in 762, 40·8%); 150 (8%) men complained of an acute yellowish-green discharge and 27 (1·4%) of a blood-stained discharge, but only eight (less than 1%) passed actual blood. Several patients, however, complained vaguely of constipation, a proportion of these having slight discomfort on defecation. Four patients had acute tenesmus and two a severe continuous pain which ceased on the overt development of perianal herpes simplex. Three patients, otherwise completely symptom free, developed acute retention of urine. A single catheterisation relieved the symptom in one of these patients but hospital admission was required for the other two. None had a stricture or any urethral infection.

Since treatment of these cases with the usual forms of therapy had a relatively high failure rate (10-27%) we decided to evaluate the treatment of male homosexually acquired rectal gonorrhoea with spectinomycin hydrochloride.

Patients and methods

DIAGNOSIS

Seventy-three episodes of rectal gonorrhoea occurring in 65 homosexual men over a period of 18 months from 1 February 1976 were treated. All patients were examined with a proctoscope and specimens were taken for microscopy and culture. The diagnosis was confirmed by the finding of Gram-negative intracellular diplococci on the smear and by the isolation and identification of *Neisseria gonorrhoeae* as described.[2]

TREATMENT

Each patient was treated with spectinomycin hydrochloride 2 g by intramuscular injection and instructed to avoid further sexual contact and to attend for follow-up examination at weekly intervals for three weeks. At each subsequent visit, repeat specimens obtained by proctoscopy were examined and all side effects relating to therapy recorded.

DETERMINATION OF MICs

Rectal isolates of *N gonorrhoeae* were collected throughout the study and preserved by snap-freezing in liquid nitrogen.[3] On completion of the study the cultures were revived and the minimum inhibitory concentrations (MICs) of spectinomycin hydrochloride determined by the agar dilution technique.[4]

Address for reprints: Dr J L Fluker, Department of Genitourinary Medicine, Charing Cross Hospital, London W6 8RF

Received for publication 21 January 1980

Results

All 65 patients were Caucasian, of whom 47 were of British origin. Their ages ranged from 18 to 67 years (mean 30·3 years). None was married. The presenting symptoms and laboratory results are shown in table I. Fifteen of the asymptomatic patients attended as a result of contact action and 10 were self-referred. Thirty-eight (58·5%) patients had had previous rectal gonorrhoea. Five patients indicated hypersensitivity to penicillin, one to streptomycin, and one to aspirin.

TREATMENT

Of the 73 separate episodes of rectal gonorrhoea treated, five cases were excluded from the analysis. Two patients received penicillin on subsequent diagnosis of latent syphilis, one developed an ischiorectal abscess requiring further treatment, one had had an ureteric diversion operation, and one developed non-specific proctitis that was treated with oxytetracycline. Fifty-six (82·4%) of the remaining 68 patients attended for one or more follow-up visits (table II).

Of those who had attended for one or more follow-up visits, three failed to be cured with spectinomycin hydrochloride. The MICs of spectinomycin hydrochloride for the preserved strains of *N gonorrhoeae* were 20 mg/l or less (mode 10 mg/l).

Discussion

Since many patients with rectal gonorrhoea are asymptomatic and unreliable in attending for follow-up, it is important for first-line treatment to be highly effective. This is supported by the recent appearance of β-lactamase-producing strains of *N gonorrhoeae* in Britain.[5]

TABLE I *Presenting symptoms of 73 episodes of male rectal gonorrhoea together with the number of patients having positive results to rectal smears cultures or both*

Symptoms	*No*	*%*
Asymptomatic	25	34·3
Rectal discharge	23	31·5
Pruritus or slight soreness	10	13·7
Low back pain	2	2·7
Blood in stool	1	1·4
Anal warts	2	2·7
Urethral discharge	2	2·7
Prolapsed haemorrhoid	1	1·4
Not recorded	7	9·6
Total	73	100·0
With positive results:		
Rectal smear	40	54·8
Rectal culture	53	72·6
Smear and culture	20	27·4

TABLE II *Outcome of treatment in 68 cases*

	No (%) of patients at follow-up visits					
Results of treatment	*1*		*2*		*3*	
Assessable	56	(82·4)	35	(51·5)	16	(23·5)
Negative result to rectal smears and cultures	54	(96·4)	33	(94·3)	16*	(100·0)
Failure	2	(3·6)	1	(2·9)	0	
Reinfection (rectal)	0		1	(2·9)	0	
Default	12	(17·6)	33	(48·5)	52	(76·5)

* One patient with a rectal infection apparently cured had acquired a fresh urethral infection

Penicillin has been less effective in treating rectal than urethral gonorrhoea in men. Fluker and Hewitt[6] reported a failure rate of 26 (27%) of 96 cases treated with intramuscular procaine penicillin (1·8 megaunits) in London, while out of 192 cases Onyeabo (personal communication, 1970) observed 18 (10·5%) failures using 3·6 megaunits of the same antibiotic supplemented with oral ampicillin 2 g and probenecid 1 g. Using intramuscular kanamycin (2 g), Fluker and Hewitt[6] found 14 (15·5%) failures among 90 cases followed in London. With co-trimoxazole in doses ranging from 16 to 20 tablets for between two and five days, Lawrence *et al*[7] reported one failure out of 15 cases, and Waugh,[8] using two tablets twice a day for seven days, reported eight (12·6%) failures out of 66 cases. There have been no reports on the effectiveness of spectinomycin hydrochloride in treating rectal gonorrhoea in male homosexuals.

Rectal gonorrhoea associated with urogenital gonorrhoea in women is common in the USA[9] and elsewhere. Duncan *et al*,[10] also in the USA, reported that spectinomycin hydrochloride in either a 2-g or 4-g dose gave a high cure rate (96%) in women with urogenital gonorrhoea, of whom 42% had additional positive rectal culture results. No significant differences have been found between the 2-g and 4-g doses of spectinomycin hydrochloride in treating urethral gonorrhoea.[11-13] The United States Center for Disease Control[14] recommends the smaller dose. Morton,[15] however, suggests that a single intramuscular dose of 4 g might delay the development of resistance, although Karney *et al*[16] found no evidence of this in 2187 cases of gonorrhoea in both sexes.

The distribution of MICs appeared to be similar to that obtained by Poster and Wood[17] for heterosexual patients and supports the finding of Platt[18] that gonococcal isolates from homosexuals are no more resistant than isolates from heterosexual patients.

In view of these findings, the discrepancy in treatment failure rates between cases of rectal and urethral gonorrhoea appears anomalous. In cases where the clinical history excludes reinfection and the dosage is sufficiently high to achieve adequate cell concentrations in vivo, bacterial interference is a putative cause. The production of antibiotic-inactivating enzymes (for example, β-lactamases) by the rectal commensals may reduce the effective concentration in situ. This mechanism seems improbable in the case of spectinomycin since enzymes active against the aminoglycosides are not liberated but remain in close association with the cytoplasmic membrane of the producer organisms.[19] The survival of gonococci in the presence of antibiotics is a function not only of the intrinsic sensitivity of the organisms but also of their physiological state,[20] and therefore other interactions between gonococci and the commensal flora could influence the outcome of treatment. These interactions may reduce the gonococcal growth rate, either by competition for nutrients or by antagonism, to below a critical level required for antibiotic activity.[21] Thus an antimicrobial agent active against both the gonococcus and a hypothetical antagonist would result in a low level of treatment failure. In this context it is interesting to note the finding of Phillips[22] that *Bacteroides fragilis* is sensitive to spectinomycin. Laboratory studies of the antimicrobial susceptibility of gonococci in mixed culture should clarify the mechanism of treatment failure in cases where the organism appears to be sensitive in vitro.

In conclusion, this study suggests that a single dose (2 g) of spectinomycin hydrochloride is an acceptable first-line treatment for rectal gonorrhoea in men, giving a cure rate of 94·5%. Caution has been advised, however, in case increased use leads to plasmid transfer[23] or transposition[19] from the enteric flora.

References

1. Fluker JL. A 10-year study of homosexually transmitted infection. *Br J Vener Dis* 1976;**52**:157-60.
2. Beck A, Fluker JL, Platt DJ. *Neisseria meningitidis* in urogenital infections. *Br J Vener Dis* 1974;**50**:367-9.
3. Ward ME, Watt PJ. The preservation of gonococci in liquid nitrogen. *J Clin Pathol* 1971;**24**:122-3.
4. Ericsson HM, Sherris JC. Antibiotic sensitivity testing: Report of an international collaborative study. *Acta Pathol Microbiol Scand (B)* 1971;suppl 217.
5. Percival A, Corkill JE, Rowlands J, *et al.* Penicillinase-producing gonococci in Liverpool. *Lancet* 1976;ii:1379-82.
6. Fluker JL, Hewitt AB. Kanamycin in the treatment of rectal gonorrhoea. *Br J Vener Dis* 1970;**46**:454-6.
7. Lawrence A, Phillips I, Nicol CS. Various regimens of trimethoprim-sulphamethoxazole used in the treatment of gonorrhoea. *J Infect Dis* 1973;**128**:suppl:673-8.
8. Waugh MA. Trimethoprim-sulphamethoxazole (Septrin) in the treatment of rectal gonorrhoea. *Br J Vener Dis* 1971;**47**:34-5.
9. Schmale JD, Martin JE, Domescik G. Observations on the culture diagnosis of gonorrhoea in women. *JAMA* 1969;**210**:312-4.
10. Duncan WC, Holder WR, Roberts DP, Knox JM. Treatment of gonorrhoea with spectinomycin hydrochloride: Comparison with standard penicillin schedules. *Antimicrob Agents Chemother* 1972;**1**:210-4.
11. Schroeter AL, Reynolds GH, Holmes KK. Spectinomycin in the treatment of gonorrhoea. *J Am Vener Dis Assoc* 1975;**1**:139-45.
12. Roberts FL. Diagnosis and treatment of acute uncomplicated gonorrhoea. *South Med Bull* 1971;**59**:18-21.
13. Cornelius CE, Domescik C. Spectinomycin hydrochloride in the treatment of uncomplicated gonorrhoea. *Br J Vener Dis* 1970;**46**:212-3.
14. Center for Disease Control. Gonorrhoea: CDC recommended treatment schedules. *Morbidity and Mortality Weekly Report* 1974;**23**:341.
15. Morton RS. *Gonorrhoea*. London; Philadelphia; Toronto: WB Saunders. 1977;190-1.
16. Karney WW, Pederson AHB, Nelson M, Adams H, Pfeifer RT, Holmes KK. Spectinomycin versus tetracycline for the treatment of gonorrhoea. *N Engl J Med* 1977;**296**:888-94.
17. Porter IA, Wood WJ. Spectinomycin minimum inhibitory concentrations for *Neisseria gonorrhoeae. Br J Vener Dis* 1974;**50**:289-90.
18. Platt DJ. Prevalence of multiple-antibiotic resistance in *Neisseria gonorrhoeae. Br J Vener Dis* 1976;**52**:384-6.
19. Davies J, Smith DI. Plasmid-determined resistance to antimicrobial agents. *Ann Rev Microbiol* 1978;**32**:469-518
20. Platt DJ, Gerken A. Inhibition of gonococci by a selective medium: disparity between isolates from sexual partners. *Br J Vener Dis* 1979;**55**:65.
21. Kraus SJ, Gellar RC, Perkins GH, Rhoden DL. Interference of *Neisseria gonorrhoeae* growth by other bacterial species. *J Clin Microbiol* 1976;**4**:288-95.
22. Phillips I. In: Phillips I, Sussman M, eds. *Infection with Non-sporing Anaerobic Bacteria*. Edinburgh: Churchill Livingstone, 1974.
23. WHO Scientific Group. *Neisseria gonorrhoeae* and gonococcal infection. *WHO Tech Rep Ser 616*. Geneva: WHO, 1978.

Br J Vener Dis 1980;**56**:400-3

Evaluation of the Papanicolaou-stained cytological smear as a screening technique for asymptomatic gonorrhoea

GAIL I GENVERT,* LEWIS M DRUSIN,*†‡ JOHN F SEYBOLT,§‖ AND DAVID W BRAUN¶

*From the Departments of †Public Health, ‡Medicine, and §Pathology, Cornell University Medical College; the *Department of Epidemiology and the ‖Papanicolaou Cytology Laboratory, New York Hospital; and the ¶Biostatistics Laboratory, Memorial Sloan-Kettering Cancer Center, New York, USA*

SUMMARY To assess the Papanicolaou smear as a screening test for asymptomatic gonorrhoea 311 women who had had simultaneous Papanicolaou smears and cervical cultures performed were studied. Of the 151 women who were culture-positive only 27 (18%) had a positive result by Papanicolaou smear. The presence of either endocervical or metaplastic cells did not increase the frequency with which gonococci were recognised. The eight cytotechnologists spent at least 30 minutes for each case looking for diplococci; the variability between observers was high.

The possibility of using an already widely accepted diagnostic technique, such as the cytological smear, as a means of screening for gonococci is very attractive. Unfortunately in this study the excessive time taken to examine the slides, the poor correlation of observers' findings, and a low sensitivity for finding diplococci made the Papanicolaou smear an impractical method for detecting gonorrhoea in asymptomatic women.

Introduction

Gonorrhoea is the most frequently reported communicable disease in the United States. During 1977, the Center for Disease Control reported 1 000 177 cases and estimated the actual incidence of gonorrhoea at 1·6-2·0 million cases annually.[1] In women, culture of samples from both the cervix and rectum on Thayer-Martin media is the recommended method for making the diagnosis of gonorrhoea.[2] Performing bacterial cultures on a large population of women at risk for this disease is both expensive and inconvenient. Public health authorities have been searching for a more practical and socially acceptable method of identifying asymptomatic cases. Recently, *Neisseria gonorrhoeae* has been recognised on Papanicolaou smears performed on women with active gonococcal infections.[3 4] These investigators have suggested that the Papanicolaou smear might be useful in detecting asymptomatic gonococcal carriers among those women who undergo an annual gynaecological examination.

In this study, the parameters that define a successful screening test (sensitivity, specificity, validity, yield, reliability, and practicality) were applied to data from women who had simultaneous cytological smears and cervical cultures performed at the New York Hospital to evaluate the Papanicolaou smear as a screening test for asymptomatic gonorrhoea.

Patients and methods

CULTURES

Cervical cultures for gonorrhoea were performed on Thayer-Martin medium according to the methods recommended by the Center for Disease Control.[5] Cervical smears were fixed in Spray-cyte (Clay-Adams) and stained according to the Papanicolaou technique.[6]

STUDY POPULATION

The study population consisted of women seen in the department of obstetrics and gynaecology at the New York Hospital who had both a Papanicolaou smear of the cervix and a cervical culture for gonorrhoea performed during a routine examination. A patient was included as a study case if her cervical culture had given a positive result for *N gonorrhoeae;*

Address for reprints: Dr L M Drusin, Department of Public Health, The New York Hospital-Cornell Medical Center, 525 East 68th Street, New York, NY 10021, USA

Received for publication 12 March 1980

however, she was included as a control if her cervical culture result was negative. Cases were identified from the gonococcal reporting forms submitted by the diagnostic microbiology laboratory at the New York Hospital to the New York City Department of Health. Controls were located from a computer printout listing all elective abortions performed at the New York Hospital. Charts on these patients were reviewed retrospectively to identify a study population consisting of an approximately equal number of cases and controls.

Names and history numbers of all these patients were arranged consecutively according to the Papanicolaou laboratory's slide accession number, which afforded a random order for the results of the gonococcal cultures.

SCREENING

Papanicolaou smears from the entire study population were reviewed in a double-blind fashion by a team of eight observers. These were licensed cytotechnologists with an average of 8·8 years of experience (range 4-15 years) of examining cytological smears at the Papanicolaou cytology laboratory. The observers were each shown good examples of smears with gonococci before the study began. They initially scanned the slides at about × 150 magnification (× 10 objective with × 15 eyepieces). If they noted cells with a collection of bacteria they switched to the high dry objective (× 40) for confirmation. This area was marked with a dot. Finally, to verify the presence of diplococci further, they examined the marked areas under oil at a magnification of × 1440. They were also asked to note the presence of metaplastic and endocervical cells in addition to diplococci. The observers were limited to 30 minutes for each case, for which there were three slides (vaginal, cervical, and endocervical). The reliability of this examination was subsequently evaluated by each of the eight observers independently reviewing the Papanicolaou smears from the same set of randomly selected 20 patients (10 cases and 10 controls).

Results

A total of 311 women who had had simultaneous Papanicolaou smears and cervical cultures performed over a 40-month period (January 1974 to May 1977) were studied. There were 151 cases and 160 controls. Cases and controls were not compared for demographic characteristics, since the results of the Papanicolaou smears and gonococcal cultures were not dependent on these factors.

The time spent screening slides for gonococci was compared with the time that cytotechnologists usually take to screen for cytological abnormalities (table I). Clearly, looking for diplococci on a Papanicolaou smear takes an inordinate amount of time. Although the observers invariably used their allotted 30 minutes for each case in their search for gonococci, they often found that they could have examined the slides for a longer period of time.

TABLE I *Time spent screening routine Papanicolaou smears compared with those for gonorrhoea*

	Routine cytology		*Gonorrhoea*
Interpretation	*% of results†*	*Time taken (mins)*	*Time taken (mins)*
Clearly negative	99·00	7·5-10*	30 (for each case)
Clearly abnormal	0·90	25	
Difficult to interpret	0·09	50	

*Calculated from the mean number of slides with negative results screened per hour by an experienced cytotechnologist
†Estimated from the total number of cases examined in one month

SENSITIVITY AND SPECIFICITY

The overall results are summarised in table II. Of the 151 women who were culture-positive for *N gonorrhoeae* only 27 (18%) had positive results on the Papanicolaou smear. Thus, the sensitivity was very low. Of the 160 women who were culture-negative for *N gonorrhoeae* all but four had results correctly identified as negative on the cytological smear—a specificity of 97%.

STATISTICAL ANALYSIS

Given the results of a standard χ^2 test, we reject the null hypothesis of no correlation between the Papanicolaou smear and the cervical culture in the detection of gonorrhoea. However, this agreement is not clinically significant. It only reflects the fact that observers regarded 90% (280/311) of all results of Papanicolaou smears reviewed as negative, giving the false impression that there is good agreement between the cytological smear and cervical culture in detecting women who do not have gonorrhoea. This, in turn, is reflected in the high specificity (97%) noted above, and it is only for this reason that our P value is significant ($P<0·001$). The Papanicolaou

TABLE II *Presence or absence of gonococci detected by Papanicolaou smears and cervical cultures*

	Cervical culture results		
Smear results	+	−	*Total*
+	27	4	31
−	124	156	280
Total	151	160	

+Positive −negative

smear as a screening test is simply not sensitive enough to pick up cases or carriers with any regularity despite a seemingly good statistical agreement between the two methods of detection.

METAPLASTIC AND ENDOCERVICAL CELLS

The sensitivity rate for each combination of presence or absence of metaplastic or endocervical cells for those smears taken from culture-positive patients is given in table III. A statistically significant difference was observed when metaplastic cells were present—that is, a sensitivity rate of 24% compared with 0% (P<0·01; Fisher's exact test). This difference is not important however because this low sensitivity is clinically unacceptable. Although there appeared to be some increased sensitivity when endocervical cells were present (27% compared with 14%), this difference was not statistically significant (P>0·1). When only slides with positive results for metaplastic cells are considered, the small difference associated with the presence of endocervical cells (29% compared with 20%) was also not statistically significant (P>0·1).

TABLE III *Percentage of Papanicolaou smears showing gonococci in relation to presence or absence of endocervical and metaplastic cells from patients with positive cervical culture results*

	% of smears showing gonococci when		
	Endocervical cells		
Metaplastic cells	+	−	*Total*
+	29 (13/45)	20 (14/69)	24 (27/114)
−	0 (0/4)	0 (0/28)	0 (0/32)
Total	27 (13/49)	14 (14/97)	18 (27/146)

+ Present − absent

RELIABILITY BETWEEN OBSERVERS

Specificity was high when the findings of the eight observers were compared with the cervical culture results (table IV). The eight cytotechnologists scored the slides from 10 culture-negative patients as positive or negative for the presence of diplococci (80 determinations). The highest specificity would have occurred if all the scores had been negative. A total of 78 (98%) negative observations was recorded. The sensitivity, on the other hand, was very low.

The eight observers scored the slides from 10 culture-positive patients as positive or negative. Of a possible 80 positive scores (highest sensitivity) only 35 (44%) positive observations were made. Again, the high specificity occurred because the observers identified the results of most cases as negative for diplococci.

TABLE IV *Correlation of the presence of diplococci on Papanicolaou smears with the results of cervical culture*

	*No of observers detecting diplococci on smears**		
Patient No	*Present*	*Absent*	*Culture results*
1	1	7	+
2	6	2	+
3	1	7	−
4	0	8	−
5	2	6	+
6	3	5	+
7	6	2	+
8	0	8	−
9	2	6	+
10	0	8	−
11	0	8	−
12	0	8	−
13	0	8	−
14	6	2	+
15	2	6	+
16	1	7	−
17	0	8	−
18	3	5	+
19	4	4	+
20	0	8	−

*All eight observers reviewed each slide independently
+ Positive − negative

Although the number of endocervical cells (EC) or metaplastic cells (MC) actually present in the smears from 10 patients with positive gonococcal culture results were not measured, it was possible to assess the agreement among the eight observers for the presence or absence of these cells in the 20 smears. If the cytotechnologists agreed perfectly, a case would be scored as either EC/MC-positive or EC/MC-negative by all eight observers. The number of cases that were scored EC-positive by zero to eight observers is given in table V. Very poor agreement was found among the observers (table V). For nine cases, four observers scored EC-positive whereas the other four scored EC-negative. The presence of metaplastic cells on the slides is also shown in table V. Although observers scored MC-positive more often than they scored EC-positive, agreement was again poor.

Discussion

Several parameters are important for evaluating a procedure as an effective screening test. Validity, which provides a good preliminary indication of the individuals who either have or do not have the disease, has two components. Sensitivity is the ability to identify correctly those who have the disease and specificity is the ability to identify correctly those who do not have the disease. In this study, sensitivity and specificity were measured by comparing an observer's skill in recognising diplococci on the Papanicolaou smears with the results of cervical

TABLE V *Variability between findings of eight observers of the presence of endocervical and metaplastic cells on Papanicolaou smears of 20 patients (10 with positive culture results for N gonorrhoeae, 10 with negative results)*

No of observers reporting presence of cells	*No of cases reported with either:*	
	Endocervical cells (EC+)	*Metaplastic cells (MC+)*
0	4*	0
1	2	0
2	2	1
3	1	4
4	9	1
5	1	3
6	0	4
7	0	3
8	1	4

+Positive
*In each of four cases, no observers reported the presence of endocervical cells (example)

cultures. Cervical culture was accepted as the definitive diagnostic procedure even though it may identify a suspected gonococcal carrier in approximately 90% of cases.[7] Our data indicated that the sensitivity of the Papanicolaou smear in identifying diplococci was only 18%. Its validity as a screening test was therefore poor.

Yield—the amount of previously unrecognised disease diagnosed and brought to treatment—is another important parameter for evaluating a screening test. The United States Public Health Service reported that, during 1977, 8 456 410 women had cultures for gonorrhoea performed in a variety of health care facilities. In this population, 393 305 (4·7%) women had positive culture results for *N gonorrhoeae*.[1] Our study could not quantitate yield; however, if the Papanicolaou smear had been used to screen these women for gonorrhoea, a sensitivity of 18% would have detected only 70 795 cases. Yield therefore would have been very low.

A screening technique must give consistent results when it is performed more than once under the same conditions on the same individual to be considered precise or reliable. In this study, variability among the eight observers who reviewed the same 20 slides was high. Thus, interobserver reliability was poor. This may be partly due to the unsuitability of the Papanicolaou stain for identifying gonococci. Since the stain is not a Gram stain, most bacteria stain a similar bluish colour, and it is not possible to distinguish between Gram-positive and Gram-negative organisms. Diplococci are also difficult to recognise because oil immersion lenses, which are necessary to identify them, are not routinely used in cytodiagnosis.

It is interesting to note that reliability between observers showed a low sensitivity even though the cytotechnologists spent an excessive amount of time searching for the diplococci (table I). The time required to screen each case was so great that this method would be impractical for detecting diplococci, even if the reliability between observers had been excellent. Thin *et al*[8] evaluated the Papanicolaou smear as a method for detecting *Candida sp.* They also concluded that the time necessary for prolonged examination of the smears was excessive.[8]

Previous investigators[3 4] have reported that the presence of metaplastic or endocervical cells or both on the smear greatly facilitated identification of the diplococci. Our data did not support this conclusion. Furthermore, our study showed that the finding of endocervical or metaplastic cells on the slides from 10 women with positive cervical culture results varied greatly among the eight cytotechnologists (table V). Although these authors[3 4] also reported that more than 50% of routine Papanicolaou smears were unsuitable for screening owing to inadequate metaplastic or endocervical cells, the eight observers at the New York Hospital did not find this a problem.

The possibility of using an already widely accepted diagnostic technique, such as the cytological smear, as a means of screening for gonococci is attractive. Unfortunately, this study suggests that the excessive time taken to examine the slides, poor interobserver reliability, and a low sensitivity for finding diplococci make the Papanicolaou smear an impractical method for detecting gonorrhoea in asymptomatic women.

We wish to thank Jane Bancroft, M Carroll Pariseau, Barbara Topf, and the cytotechnologists of the Papanicolaou Cytology Laboratory for their assistance.

References

1. US Department of Health Education and Welfare. *STD Fact Sheet*, 34th edn. HEW Publication No 79-8195, Atlanta, Georgia: CDC, 1979;2:34.
2. Schmale JD, Martin jun JE, Domescik G. Observations on the culture diagnosis of gonorrhea in women. *JAMA* 1969;**210**:312-4.
3. Arsenault GM, Kalman CF, Sorensen KW, Craig JD. The Papanicolaou smear as a technique for gonorrhea detection: a feasibility study. *J Am Vener Dis Assoc* 1976;**2**:35-8.
4. Heller CJ. *Neisseria gonorrhoeae*' in Papanicolaou smears. *Acta Cytol* 1974;**18**:338-40.
5. Lynnette EH, Spaulding EH, Truant JP. *Manual of Clinical Microbiology*. 2nd edn. Washington DC: American Society of Microbiology, 1974:124-9.
6. Papanicolaou GN. *Atlas of Exfoliative Cytology*. Cambridge, Mass: Harvard University Press, 1954:8.
7. Caldwell JG, Price EV, Pazin GJ, Cornelius III CE: Sensitivity and reproducibility of Thayer-Martin culture medium in diagnosing gonorrhea in women. *Am J Obstet Gynecol* 1971;**109**:463-8.
8. Thin RNT, Atia W, Parker JDJ, Nicol CS, Canti G. Value of Papanicolaou-stained smears in the diagnosis of trichomoniasis, candidiasis, and cervical herpes simplex virus infection in women. *Br J Vener Dis* 1975;**51**:116-8.

Br J Vener Dis 1980;**56**:404-7

Prevalence of antichlamydial antibody in London blood donors

S DAROUGAR,* T FORSEY,* D A BREWERTON,† AND K LL ROGERS‡

*From the *Department of Clinical Ophthalmology, Institute of Ophthalmology; the †Department of Rheumatology, Westminster Hospital; and the ‡South London Blood Transfusion Centre, London*

SUMMARY The prevalence of type-specific antichlamydial antibody in a population of blood donors in London was studied using a microimmunofluorescence test. Twenty-six (17%) of 150 women and 38 (26%) of 150 men had antichlamydial antibody (IgG at ⩾1/16 or IgM ⩾1/8 or both). Of these, five (3%) women and one (0·75%) man had this antibody directed against *Chlamydia trachomatis* serotypes D-K, responsible for genital infections, and one man had antibody to *Chlamydia psittaci* agents. The remaining 57 men and women had antibody against an atypical chlamydial isolate designated *Chlamydia* IOL-207, which is iodine-negative and serologically distinct from both *C trachomatis* and *C psittaci.* The nature and location of infection by this agent are obscure.

The results of this study suggest that the prevalence of sexually transmitted infection with *C trachomatis* serotypes D-K in a normal adult population in London is very low.

Introduction

Chlamydial genital infection is a common sexually transmitted disease. Chlamydia have been isolated from up to 68% of men with nongonococcal urethritis (NGU),[1] from up to 62% of women with gonorrhoea,[2] and from up to 31% of unselected women attending a sexually transmitted diseases clinic.[2] Ocular chlamydial infection of sexually transmitted origin—namely, TRIC ophthalmia neonatorum, inclusion conjunctivitis, and TRIC punctate keratoconjunctivitis—is also common,[3] and evidence is accumulating that *Chlamydia trachomatis* may cause pneumonitis,[4] pelvic inflammatory disease,[5 6] perihepatitis, peritonitis,[7] and endocarditis.[8]

The rate of exposure to chlamydial agents, particularly to *Chlamydia trachomatis,* in communities is not known. In San Francisco[9] an isolation rate of 11% was reported in women attending a birth control clinic, and in Seattle[10] the chlamydial isolation rate in pregnant women having routine examinations was as high as 12·7%. In this report we present the results of a study on the presence and concentrations of type-specific antichlamydial antibodies in a group of adult men and women blood donors in South London.

Subjects and methods

One hundred and fifty male and 150 female blood donors were selected from a population of volunteers attending a South London transfusion centre with a catchment area including Kent, Sussex, Surrey, and London. Their mean ages were 38 ± 14 and 40 ± 13 years respectively. The volunteers were apparently free from any major chronic diseases, were not under treatment, were feeling healthy at the time of blood donation, and were not known to be in contact with any infectious disease. Volunteers with overt herpes simplex virus infection or with known chronic urogenital infections requiring treatment were excluded.

Blood was collected by venepuncture; the serum was separated and kept at −20°C for 2-4 months until tested.

MICRO-IF TEST

The modified microimmunofluorescence (micro-IF) test of Treharne *et al*[11] was used to detect antichlamydial antibodies in serum samples. These samples were screened for antichlamydial IgG and IgM at dilutions starting at 1/16 and 1/8 respectively. Goat anti-human IgG and IgM fluorescein-labelled conjugates (Hyland Division, Travenol Laboratories)

Address for reprints: Dr S Darougar, Institute of Ophthalmology, Judd Street, London WC1H 9QS

Received for publication 23 April 1980

TABLE I *Type-specific antichlamydial antibody in sera from 150 female and 150 male blood donors*

	No of sera giving positive results			
	Women		*Men*	
Chlamydial species	*No*	*%*	*No*	*%*
C trachomatis				
Serotypes A-C	0		0	
Serotypes D-K	5	3	1	0·75
LGV serotypes 1-3	0		0	
C psittaci				
Representatives[11]	0		1	0·75
C IOL-207 (atypical)	21	14	36	24
All species	26	17	38	26

were used at a dilution of 1/20 for IgG and 1/10 for IgM. Rhodamine conjugated bovine serum albumin (Difco Laboratories) at a dilution of 1/40 was used as a counterstain. Sera were considered type-specific when the titre of antibody against a single antigen pool was at least twofold higher than against any other cross-reacting antigen.[11] Other technical details of the micro-IF test have been described elsewhere.[11 12]

Results

Antichlamydial antibody at any concentration was detected in 17% of women and 26% of men (table I and figure). Antichlamydial IgG (at titres $\geq$1/16) against *C trachomatis* serotypes D-K (responsible for genital and ocular infection) was detected in five (3%) women and one (0·75%) man. Antichlamydial IgM was detected in only one woman, and this was also directed against *C trachomatis* D-K. This patient also had IgG at a titre of 1/64 against serotypes D-K.

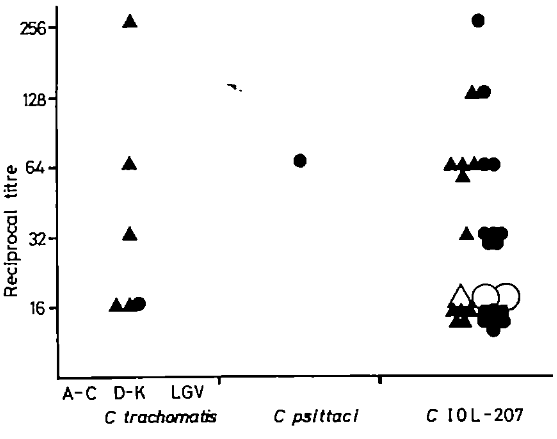


FIGURE *Distribution of titres of type-specific antibody in sera from 150 female and 150 male blood donors.*

If IgG at a titre $\geq$1/64 and IgM at a titre $\geq$1/8 is taken as indicative of a current chlamydial infection,[13] two women showed these significant micro-IF results against serotypes D-K.

Antichlamydial IgG at a titre of 1/64 against representatives of *Chlamydia psittaci* was detected in the serum of one man. No antibody was detected against *C trachomatis* serotypes A-C (responsible for hyperendemic trachoma) or the LGV serotypes.

Antichlamydial IgG (at a titre $\geq$1/16) against TRIC/IR/IOL-207/OT (IOL-207), an atypical chlamydial isolate,[12 14] was detected in 21 (14%) women and 36 (24%) men. In nine of them IgG was detected at a titre $\geq$1/64 (figure). No IgM antibody was detected against *C* IOL-207. The cross-reactivity of sera showing antibodies against *C* IOL-207 and *C trachomatis* serotypes are shown in table II.

Discussion

Cell culture systems for isolation of chlamydia[15-18] are sensitive for the diagnosis of chlamydial infections. Because of their technical complexities, however, and the particular requirements for collection and transport of specimens,[19] these tests are not considered to be practicable for use in small diagnostic laboratories or in epidemiological surveys of chlamydial infections.

The modified micro-IF test used in the present study is a sensitive, simple, and rapid serological test for the detection of type-specific antichlamydial IgG, IgM, or IgA in whole blood, sera, or local discharges. Detection of type-specific antibody after screening of blood against four antigen pools which correspond with each of the four main clinical and epidemiological types of chlamydial infections—that is, hyperendemic trachoma (*C trachomatis* serotypes A-C), ocular and genital infections of sexually transmitted origin (*C trachomatis* serotypes D-K), lymphogranuloma venereum (*C trachomatis* LGV agents 1-3), and *C psittaci* infections (representative isolates of *C psittaci*)—may indicate which clinical and epidemiological type of infection is implicated.

In the present study, antibody against *C trachomatis* serotypes D-K was detected in five (3%) of 150 of the women and in one (0·75%) of 150 of

TABLE II *Cross-reactivity of 57 sera showing IgG antibody against Chlamydia IOL-207*

IgG titre against C trachomatis serotypes	*IgG titre against C IOL-207*				
	16	*32*	*64*	*128*	*256*
0	43	5	5		
16			1	1	
32				1	
64					1

the men tested. Studies by Treharne *et al*[11] showed that in women with non-specific cervicitis the percentage of chlamydial isolation-positive patients with antibody titres $\geqslant 1/8$ was 77% whereas only 14% of isolation-negative patients showed antibody at this titre or higher. In men with non-specific urethritis, the percentage of chlamydial isolation-positive patients with antibody at this titre was 51% whereas in isolation-negative patients antibody was detected in only 10%. These findings may suggest that the micro-IF positivity rate in women corresponds with the results of cell culture for defining chlamydial infection, whereas the micro-IF positivity rate in men may underestimate the frequency of the infection by approximately 50% in comparison with the cell culture system.

The prevalence of infection with *C trachomatis* D-K serotypes obtained in our group of healthy adults from South London is markedly lower than that obtained in Seattle, USA, where antibody against *C trachomatis* D-K was detected by a micro-IF test in 26% of the normal adult population.[20] This marked difference in prevalence, however, may partly reflect different patterns of sexual activity and the socioeconomic status of the people included in the two investigations and may be partly due to the testing of sera at the lower dilution of 1/8 by the workers in Seattle.

In the present study antibody against the chlamydial agent IOL-207 was detected in the blood of 14% of female and of 24% of male blood donors. *C* IOL-207 is an atypical iodine-negative chlamydial isolate obtained from the eye of an Iranian child with trachoma using an egg-culture system. We found that this agent has certain properties in common with *C trachomatis* and *C psittaci* but is serologically distinct from both of these chlamydial species (unpublished data). Experimental inoculation of a baboon[14] demonstrated the pathogenicity of this organism for the conjunctiva and urethra. During this experimental work we found that cell culture is not sensitive for reisolation of *C* IOL-207 but that the organism can be reisolated in egg culture if several passages were attempted (unpublished data). The high frequency of antibody against *C* IOL-207 detected in our group of blood donors and in the blood of patients reported by Dwyer *et al*[12] may suggest that infection by *C* IOL-207 is rather common in certain communities. Because of the apparent insensitivity of the cell culture systems commonly in use for isolating chlamydia, however, the nature and location of infection caused by this agent is still obscure.

The pattern of cross-reactions obtained by the micro-IF test when sera positive (at a titre $\geqslant 1/16$) for *C* IOL-207 are reacted against *C trachomatis* antigens is shown in table II. As may be observed, if the *C* IOL-207 antigen was excluded from this test four out of 57 sera would have been falsely recorded as having antibody titres $\geqslant 1/16$ to *C trachomatis* alone. We have also found that more cross-reactions occur between all chlamydial antigens when lower dilutions of sera are tested (unpublished data).

C psittaci agents are commonly pathogenic for mammals and birds and occasionally may infect man. The presence of antibody against *C psittaci* antigens in one male blood donor may suggest an infection with one of the various *C psittaci* agents, including psittacosis infection.

This study was supported by grants from the Department of Health and Social Security and an anonymous donor.

References

1. Alani MD, Darougar S, Burns DC, Thin RN, Dunn H. Isolation of *Chlamydia trachomatis* from male urethra. *Br J Vener Dis* 1977;**53**:88-92.
2. Hilton AL, Richmond SJ, Milne JD, Hindley F, Clarke SKR. *Chlamydia* A in the female genital tract. *Br J Vener Dis* 1974;**50**:1-10.
3. Anonymous. Chlamydial infections of the eye. *Lancet* 1977;ii:857-8.
4. Beem MO, Saxon EM. Respiratory tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. *N Engl J Med* 1977;**296**:306-10
5. Mårdh P-A, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977;**296**:1377-9.
6. Treharne JD, Ripa KT, Mårdh P-A, Svensson L, Weström L, Darougar S. Antibodies to *Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis* 1979;**55**:26-9.
7. Müller-Schoop JW, Wang S-P, Munzinger J, Schlapfer HU, Knoblauch M, Ammann RW. *Chlamydia trachomatis* as a possible cause of peritonitis and perihepatitis in young women. *Br Med J* 1978;i:1022-4.
8. Van der Bel-Kahn JM, Watanakunokorn C, Menefee MG, Long HD, Dicter R. *Chlamydia trachomatis* endocarditis. *Am Heart J* 1978;**95**:627.
9. Schacter J. Chlamydial infections. *N Engl J Med* 1978;**298**:490-5.
10. Alexander ER, Chandler J, Pheifer TA, Wang S-P, English M, Holmes KK. Prospective study of perinatal *Chlamydia trachomatis* infection. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977:148-52.
11. Treharne JD, Darougar S, Jones BR. Modification of the micro-immunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977;**30**:510-7.
12. Dwyer RStC, Treharne JD, Jones BR, Herring J. Chlamydial infection. Results of micro-immunofluorescence test for the detection of type-specific antibody in certain chlamydial infections. *Br J Vener Dis* 1972;**48**:452-9.
13. Treharne JD, Darougar S, Simmons PD, Thin RN. Rapid diagnosis of chlamydial infections of the cervix. *Br J Vener Dis* 1978;**54**:404-8.
14. Darougar S, Monnickendam MA, El-Sheikh H, Treharne JD, Woodland RM, Jones BR. Animal models for the study of chlamydial infections of the eye and genital tract. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977:148-52.

15. Darougar S, Kinnison JR, Jones BR. Simplified irradiated McCoy cell culture for isolation of chlamydiae. In: Nichols RL, ed. *Trachoma and Related Disorders.* Amsterdam: Excerpta Medica, 1971:63-70.
16. Kuo C-L, Wang S-P, Wentworth BB, Grayston JT. Primary isolation of TRIC organisms in HeLa cells treated with DEAE-Dextran. *J Infect Dis* 1972;**125:**665-8.
17. Wentworth BB, Alexander ER. Isolation of *Chlamydia trachomatis* by use of 5-iodo-2-deoxyuridine treated cells. *Appl Microbiol* 1974;**27:**912-6.
18. Ripa KT, Mårdh P-A. New simplified culture technique for *Chlamydia trachomatis.* In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:323-7.
19. Darougar S, Jones BR, Kinnison JR, Vaughan-Jackson JD, Dunlop EMC. Chlamydial infection. Advances in the diagnostic isolation of chlamydia, including TRIC agents, from the eye, genital tract and rectum. *Br J Vener Dis* 1972;**48:**416-20.
20. Wang S-P, Grayston JT, Kuo C-C, Alexander ER, Holmes KK. Serodiagnosis of *Chlamydia trachomatis* infection with the micro-immunofluorescence test. In: Hobson D, Holmes KK, eds. *Nongonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977:237-48.

Br J Vener Dis 1980;**56**:408-11

Treatment of vulvovaginal candidal infection with miconazole-coated tampons

N A M BERGSTEIN

From the Department of Obstetrics and Gynaecology, Regional Hospital, Zevenaar, the Netherlands

SUMMARY In an evaluation of three different treatment regimens miconazole-coated tampons proved to be effective for the relief of vulvovaginal candidal infection. A mycological and clinical cure rate of about 93% was achieved in patients treated with one tampon twice daily (up to a total of 15 tampons); 61% of patients were mycologically cured with a regimen of one tampon daily for only five days. Reinfection rates were less than 4% with all three regimens. The tampons were well accepted by all the patients.

Introduction

Although there is a variety of antifungal compounds available for the treatment of candidal vulvovaginitis[1] it is clear that the clinical efficacy of such agents may be offset in many patients who fail to complete a full course of treatment.[2] For many years most vaginal antifungal agents have been routinely prescribed for 14 days or more, but such long courses of treatment may be abandoned prematurely by patients who experience rapid symptomatic relief of their vaginitis[2] or by those who object to the usual creams, ointments, pessaries, or tablets because of difficulty of application or leakage or for other aesthetic reasons.[3-5]

One compound, miconazole, has now been made available in the form of a coating on absorbent vaginal tampons. This presentation should be more aesthetically acceptable to patients than vaginal creams or pessaries; however the clinical efficacy of miconazole-coated tampons requires evaluation.

This paper presents the clinical and mycological findings of a study of miconazole-coated tampons in the treatment of patients with candidal vaginitis and compares the relative efficacies of three different regimens.

Patients and methods

STUDY POPULATION

The patients admitted to the study were all premenopausal patients attending the obstetric and gynaecological outpatient clinic with features of vaginal candidosis—such as discharge, pruritus, dysuria, and dyspareunia—which was confirmed twice by the presence of *Candida* species on phase contrast microscopy and on culture of vaginal swabs on Nickerson's medium (Ortho Pharmaceutica Co, Belgium). (The latter is a selective medium on which members of the genus *Candida* grow as dark-brown or black colonies.) Trichomonads were excluded by negative results on phase contrast microscopy of vaginal swabs.

TREATMENT REGIMENS

Group A

All patients visiting the outpatient clinic between October 1976 and February 1977 were treated with a total of 15 medicated tampons coated with miconazole 100 mg in an inert base (Janssen Pharmaceutica, Belgium), one inserted into the vagina twice daily, up to a total of 15 tampons.

Group B

All patients attending between July and August 1977 were treated with 10 miconazole tampons, one inserted into the vagina twice daily for five days.

Group C

All patients attending between September and November 1977 were treated with a total of only five tampons, one inserted daily for five days.

The number of patients in each group, their ages, and clinical factors that may have predisposed to candidal infection are summarised in table I. Although the groups were well matched for age, there was only one pregnant patient in group C and only 3% of patients in group B were fitted with an intrauterine contraceptive device compared with 13%

Address for reprints: Dr N A M Bergstein, Department of Obstetrics and Gynaecology, Outpatient Clinic, Streekziekenhuis, Zevenaar, the Netherlands

Received for publication 12 February 1979

TABLE I *Clinical histories of patients treated with miconazole-coated tampons*

Clinical details	*Treatment regimen* *Group A**	*Group B†*	*Group C‡*
No of patients	83	58	90
Median age (years)	30	30	30
Age range (years)	17-48	18-55	19-48
No pregnant	10	8	1
No using oral contraceptives	28	27	53
No with intrauterine contraceptive device	11	2	12
No previously treated with anticandidal therapy	7	4	0
No previously treated with trichomonacides	0	0	1

*Treated twice daily for 7½ days
†Treated twice daily for five days
‡Treated daily for five days

in groups A and C; this latter difference was not statistically significant.

No patients had recently used antibiotics and none had diabetes mellitus. No medications were prescribed for any of the patients apart from the miconazole-coated tampons.

FOLLOW-UP

Follow-up examinations were performed on patients in all three groups 3-7 days after the end of treatment; for patients who were mycologically cured at this time a second follow-up examination was carried out one month after the end of treatment to determine rates of recurrence of candidosis.

On admission to the study and at subsequent follow-up examinations the patients were assessed clinically for severity of the following signs and symptoms; vaginal discharge, vulval pruritus, dysuria, dyspareunia, and vulval erythema. These features were rated as: "absent," "mild," or "more or less severe." A vaginal swab was also taken at each examination for culture of *Candida* species.

STATISTICS

Statistical evaluation of the differences in mycological cure rates between groups A, B, and C was performed using the χ^2 test (2×2 contingency table, two-tailed) with Yates's correction.

Results

CURE RATES

About 93% of patients in groups A and B had become culture-negative for *Candida* 3-7 days after treatment with miconazole-coated tampons (table II); however the mycological cure rate for patients in group C was only 61%. The differences in mycological cure rates between groups A and C ($\chi^2 = 22{\cdot}216$, P<0·0001) and between groups B and C ($'^2 = 16{\cdot}9886$, P<0·0001) were statistically significant. In all three groups patients who became *Candida*-negative remained so; mycological recurrence rates were less than 4% for all three groups (table II).

RELIEF OF SYMPTOMS

The high mycological cure rates in groups A and B were reflected by high rates of relief from symptoms of candidosis (table III). On admission almost all the patients in these groups had "more or less" severe discharge and pruritus, but the frequency of these symptoms was reduced to less than 9% 3-7 days after treatment, with only low rates of recurrence or persistence of symptoms among mycologically cured patients assessed one month after treatment. Among patients in group C the reduction in frequency of vaginal discharge was less impressive than for groups A and B, although the relief of pruritus was similar to that for groups A and B. The other symptoms and signs of vaginal candidosis were noted less often than pruritus and discharge at the time of admission to the study; they were relieved successfully for all but a few patients with all three treatment regimens.

SUBJECTIVE ASSESSMENT

The miconazole-coated tampons were well accepted by all the patients. More than 80% of patients in group A and 69% of patients in group B subjectively described the results of their treatment as excellent,

TABLE II *Mycological findings in three groups of patients treated for vaginal candidosis with miconazole-coated tampons*

	Group A			*Group B*			*Group C*		
Time of sample	*No of cases*	*No with negative cultures*	*% of total*	*No of cases*	*No with negative cultures*	*% of total*	*No of cases*	*No with negative cultures*	*% of total*
Before treatment	83	0	0·0	58	0	0·0	90	0	0·0
3-7 days after treatment*	83	77	92·8	58	54	93·1	90	55	61·1
1 month after treatment† ‡	77	75	97·4	53§	52	98·1	55	53	96·4

*A *vs* B = not significant; A *vs* C = χ^2_1 22·216, P<0·0001; B *vs* C = χ^2_1 16·9886, P<0·0001
†A *vs* B = not significant; A *vs* C = not significant, B *vs* C = not significant
‡Patients examined one month after treatment were only those who were mycologically negative 3-7 days after treatment
§One patient did not attend for the second follow-up visit

TABLE III *Relief of signs and symptoms of vaginal candidosis in three groups of patients treated with miconazole-coated tampons*

		Group A		Group B		Group C	
Sign or symptom	*Time of examination (in relation to treatment)*	*No of cases*	*% with score 2**	*No of cases*	*% with score 2**	*No of cases*	*% with score 2**
Vaginal discharge†	Before	83	98·8	58	100·0	90	83·3
	3-7 days after	83	8·4	58	3·4	90	13·3
	1 month after	77	5·2	53	0·0	55	0·0
Vaginal discharge‡	Before	83	100·0	58	100·0	90	80·0
	3-7 days after	83	8·4	58	1·7	90	13·3
	1 month after	77	5·2	53	0·0	55	0·0
Vulval pruritus	Before	83	97·6	58	84·5	90	44·4
	3-7 days after	83	6·0	58	3·4	90	2·2
	1 month after	77	5·2	53	0·0	55	1·8
Dysuria	Before	83	81·9	58	72·4	90	16·7
	3-7 days after	83	6·0	58	3·4	90	0·0
	1 month after	77	3·9	53	0·0	55	0·0
Dyspareunia	Before	83	31·3	58	24·1	90	11·1
	3-7 days after	83	1·2	58	1·7	90	0·0
	1 month after	77	1·3	53	0·0	55	0·0
Vulval erythema	Before	83	1·2	58	0·0	90	0·0
	3-7 days after	83	0·0	58	0·0	90	0·0
	1 month after	77	0·0	53	0·0	55	0·0

*Severity scores: 0 = absent, 1 = slight, 2 = more or less severe
†Patient's own assessment
‡Investigator's assessment

and their opinions were matched by the overall assessments of the investigator (table IV). In group C only half the patients considered the treatment as excellent. The patients noted complete disappearance of their symptoms five days after the beginning of treatment in 61%, 79%, and 27% of cases in groups A, B, and C respectively.

Discussion

The results of this study demonstrate that miconazole-coated tampons are effective in the relief of vulvovaginal candidal infection provided they are used twice daily for at least five days. The high clinical and mycological cure rates achieved with this regimen compare favourably with published cure rates for miconazole creams and pessaries used for longer periods[1 6] and with the results of the first interim analysis of a multicentre evaluation of the miconazole-coated tampon in Britain.[7] Furthermore the tampons are a highly acceptable mode of presentation for vaginal antifungal compounds; they could be used easily and continue to be used during menstruation.

TABLE IV *Subjective overall assessment of treatment of vaginal candidosis with miconazole-coated tampons*

	Patient's assessment			*Investigator's assessment*		
Effect of therapy	*Group A*	*Group B*	*Group C*	*Group A*	*Group B*	*Group C*
Excellent	70	40	48	69	40	47
Good	3	8	21	5	5	20
Doubtful	6	7	18	5	10	20
Failure	3	0	0	3	0	0
No opinion	1	3	3	1	3	3

The comparatively poor therapeutic success of the use of one tampon daily for five days was a notable feature of this trial. From a retrospective analysis of data from large numbers of published trials of vaginal antifungal agents, it appeared that a 14-day course of treatment was the minimum period needed to ensure high cure rates with low recurrence of candidosis. The present study, together with trials of short courses of imidazole-derivative antifungal agents,[8-10] shows that three-day or five-day courses may be as effective as 14-day courses in many cases. Comparison of the data for groups B and C of the present study however suggests that it may be the total dose rather than the time during which the dose is given which is the important factor.

Future studies should be designed to investigate the relationship between the total antifungal dose, duration of therapy, and therapeutic success in order to establish optimal conditions for the topical treatment of vaginal candidosis.

References

1. Odds FS. Cure and relapse with antifungal therapy. *Proc Roy Soc Med* 1977;70, suppl 4:24-8.
2. Masterton G, Henderson J, Napier I, Moffett M. Vaginal candidosis. *Br Med J* 1976;i:712-3.
3. Henderson JN, Tait IB. The use of povidone-iodine (Betadine) pessaries in the treatment of candidal and trichomonal vaginitis. *Curr Med Res Op* 1975;3:157-62.

4. Higton BK. A trial of clotrimazole and nystatin in vaginal moniliasis. *Journal of Obstetrics and Gynaecology of the British Commonwealth* 1973;**80**:992-5.
5. Weuta H. Clotrimazol-vaginaltabletten—klinische Prüfung im offenen versuch. *Arzneim Forsch* 1972;**22**:1291-4.
6. Sawyer PR, Brogden RN, Pinder RM, Speight TM, Avery GS. Miconazole: a review of its antifungal activity and therapeutic efficacy. *Drugs* 1975;**9**:406-23.
7. Donald WH. A study on the cure and relapse of candidal vaginitis with a novel formulation of miconazole. In: *Candidal Vaginitis*. London: Academic Press Inc and the Royal Society of Medicine. *International Congress and Symposium Series* 1979;**7**:31-3.
8. Balmer JA. Three-day therapy of vulvovaginal candidiasis with econazole: a multicentre study comprising 996 cases. *Am J Obstet Gynecol* 1976;**126**:436-41.
9. Masterton G, Napier IR, Henderson JN, Roberts JE. Three-day clotrimazole treatment in candidal vaginitis. *Br J Vener Dis* 1977;**53**:126-8.
10. Bergstein NAM, Nuÿten STM. Treatment of vulvovaginal candidiasis with miconazole tampons and miconazole cream: a comparative trial. *Tÿdschrift voor Geneesmiddelonderzoele,* 1980; in press.

Br J Vener Dis 1980;56:412-3

Further evidence of the efficacy of co-trimoxazole in granuloma venereum*

SARDARI LAL AND B R GARG

From the Department of Dermatology and Sexually Transmitted Diseases, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India

SUMMARY One hundred and sixteen patients with granuloma venereum were treated with co-trimoxazole two tablets twice daily. All the patients responded; most of them required treatment for 10-15 days. Only two patients had recurrences, which were probably due to reinfections. No side effects of the drug occurred. Co-trimoxazole is considered to be the treatment of choice for granuloma venereum because of its high efficacy, few side effects, easy administration, and absence of any risk of masking concomitant syphilis.

Introduction

The efficacy of co-trimoxazole in the treatment of granuloma venereum was studied in 10 cases by Garg *et al.*[1] In the present paper the results of treatment of a further 116 patients are presented.

Patients and methods

One hundred and sixteen patients (84 male and 32 female) attending the Department of Dermatology and Sexually Transmitted Diseases, Jipmer Hospital, Pondicherry, between February 1978 and August 1979, were diagnosed as having granuloma venereum by the demonstration of Donovan bodies and included in the study.

The duration of the disease was less than 30 days in 37 patients, between 31 days and one year in 71, and more than a year in eight.

TREATMENT

All the patients were given two tablets of co-trimoxazole twice daily by mouth (each tablet containing trimethoprim 80 mg and sulphamethoxazole 400 mg). Lesions were noted and smears for Donovan bodies repeated on the third, fifth, and seventh days of treatment. Patients were observed for any side effects.

FOLLOW-UP

When the lesions were healed the patients were discharged and asked to come back for examination once a month. Patients who did not reattend were recalled by post at least twice.

Results

Donovan bodies disappeared from tissue smears after treatment for five days and the lesions healed after treatment for 7-22 days in all cases. The mean duration of treatment required was about 12·5 days; 16 patients required treatment for 7-9 days, 92 for 10-15 days, and eight for more than 15 days.

One patient developed jaundice after 11 days' treatment. Liver function tests showed total serum bilirubin 3·2 mg/100 ml (54·7 μmol/l), direct serum bilirubin 2·2 mg/100 ml (37·6 μmol/l), SGOT 68 units, SGPT 78 units, and serum alkaline phosphatase 22 units. Co-trimoxazole was stopped and the patient recovered from his hepatitis; the genital lesions healed without further treatment. This complication was most likely due to a coincidental viral hepatitis. Apart from this incident no other side effects were seen in the other patients.

Forty-five patients attended for follow-up examination; of these 43 did not show any evidence of recurrence. One patient, whose lesions recurred two months after treatment, may have been reinfected by his wife, who had not been treated. The remaining patient defaulted and the cause of recurrence could not be determined.

Discussion

All the 116 patients responded to treatment with co-trimoxazole; the majority (92) required treatment for

---

*Paper read at the Fourth National Conference of the Indian Association for the Study of Sexually Transmitted Diseases, Visakhapatham, 23-24 February 1980

Address for reprints: Dr S Lal, Department of Dermatology and STD, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India

Received for publication 13 March 1980

10-15 days. Only two of these patients had a recurrence of the disease about two months after treatment. Thus the drug is highly effective for the treatment of granuloma venereum.

Co-trimoxazole appears to be as effective as, if not more so than, streptomycin or tetracycline. It shares with streptomycin the advantage of not masking a concomitant early syphilitic infection.[2] Side effects of co-trimoxazole were negligible compared with those of streptomycin, which produces giddiness in about 16% of patients with the dosage used for this disease.[3] Another advantage is that it is also effective against gonorrhoea[4] and lymphogranuloma venereum,[5] whereas streptomycin is not effective against either of these diseases. Lastly, ease of administration of the drug is another advantage over streptomycin, for which twice daily injections are required.

Co-trimoxazole can thus be considered to be the drug of choice for the treatment of granuloma venereum in a dose of two tablets twice daily for 10-15 days. Its higher cost compared with that of streptomycin hinders its recommendation as the routine treatment for the disease.

We are grateful to the Director of our Institute for permission to report these findings. Our thanks are also due to the residents, laboratory technician, and social worker of the department for their help in the study.

References

1. Garg BR, Lal S, Sivamani S. Efficacy of co-trimoxazole in Donovanosis. *Br J Vener Dis* 1978;**54**:348-9.
2. Svindland HB. Treatment of gonorrhoea with sulphamethoxazole-trimethoprim. Lack of effect on concomitant syphilis. *Br J Vener Dis* 1973;**49**:50-3.
3. Lal S. Continued efficacy of streptomycin in the treatment of granuloma inguinale. *Br J Vener Dis* 1971;**47**:454-5.
4. Sowmini CN, Vijayalakshmi K, Rao GC, Gopalan KN, Kumaraswamy E. Septrin in the treatment of gonorrhoea. *Indian J Dermatol Venereol Lepr* 1976;**42**:110-2.
5. Chandrasekaran NK. Efficacy of trimethoprim-sulphamethoxazole combination in the treatment of lymphogranuloma venereum. *Bulletin of the South-east Asian Western Pacific Region Branch of the International Union against Venereal Diseases and the Treponematoses* 1976;**1**:21-2.

Br J Vener Dis 1980;**56**:414-5

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Conjunctivitis due to *Haemophilus ducreyi* infection

Sir,

Haemophilus ducreyi is an extremely rare cause of ocular infections.[1] This organism, which was described by Ducrey in 1889, is the aetiological agent of soft chancre or chancroid—a venereal disease. The natural reservoir of this small pleomorphic Gram-negative bacillus is the genitourinary tract of man. In civilian populations this bacterium may be responsible for up to 10% of reported cases of chancroid.[2]

A 25-year-old male medical student complained of acute conjunctivitis within four days of performing vaginal examinations on five female patients. His chief complaint was a pulsating ache and erythema of the conjunctiva of the left eye. There was also puffiness and tenderness of the upper lid of this eye and pain on attempted use of the extraocular muscles of the eye. A profuse yellow mucoid exudate was present in the medial punctum of the eye.

On closer examination it was noted that the scleral blood vessels were dilated with haemorrhage from some of these vessels. An enlarged non-tender left preauricular lymph node and a slightly enlarged non-tender submaxillary gland were detected by palpation. A presumptive diagnosis of epidemic keratoconjunctivitis, probably due to adenovirus, was made based on the clinical picture presented.

Approximately one hour after examination by an ophthalmologist and before treatment with an ophthalmic solution (naphazoline HCl in a dosage of 2-3 drops) into the infected eye in conjunction with cold compresses to reduce swelling a portion of the exudate was collected for culture using a cotton swab. A sheep-blood agar plate and a chocolate agar plate were inoculated and both incubated in a candle jar for 24 hours at 37°C. After this period a few identical tiny greyish translucent colonies were observed on both media; however, more colonies appeared on the chocolate agar than on the blood agar plate. A Gram stain of a few colonies from both media showed pleomorphic Gram-negative bacilli, morphologically consistent with the genus *Haemophilus*.

Three colonies from each medium were inoculated on to separate trypticase soy agar (TSA) plates; a V factor (nicotinamide adenine nucleotide) strip and an X factor (hemin) strip (Baltimore Biological Laboratory, Cockeysville, Maryland, USA) were placed on both plates. The plates were incubated under CO_2 tension in a candle jar at 37°C and examined after 24 hours.

Three colonies were also transferred to a test tube containing 10 ml of nitrate broth (Difco Laboratories, Detroit, Michigan, USA) and incubated for 24 hours at 37°C. The TSA plates showed tiny refractile colonies growing around the X factor strip; no growth occurred around the V factor strip. No change was observed in the nitrate broth indicating that the organism did not reduce nitrate to nitrite. The organism was identified as *Haemophilus ducreyi.*

After 11 days' treatment with the ophthalmic solution and cold compresses the patient became asymptomatic and has not experienced any recrudescence.

Yours faithfully,

J E Gregory
R W Henderson
R Smith

Departments of Microbiology and
Ophthalmology,
Howard University Hospital and
College of Medicine,
Washington DC,
USA

References

1. Fedukowicz HB. *External Infections of the Eye.* 2nd ed. New York: Appleton-Century-Crofts, 1978:160-1.
2. Joklik WK, Willett HP. *Zinsser Microbiology*, 16th ed. New York: Appleton-Century-Crofts, 1976:471-8.

TO THE EDITOR, *British Journal of Venereal Diseases*

Trichomonal urethritis in men

Sir,

I would like to offer a comment on your recent article[1] on the investigation of trichomonal urethritis in men. The technique was shown to be sensitive and capable of demonstrating small numbers of trichomonads in a urine specimen. However, when 18 of 21 male contacts of women known to have trichomonal vaginitis produced negative culture results the only conclusions offered were "that most male contacts shed relatively few trichomonads and that the infective dose for women must be correspondingly small."

May I suggest as an alternative, and more likely, conclusion that trichomonads were not found in these 18 men because they were not present in the first place.

This report would appear to confirm my suggestion[2] that trichomonal vaginitis is not (necessarily) a sexually transmitted disease.

Yours faithfully,

N Rosedale

Genitomedical Department,
Hillingdon Hospital,
Uxbridge, Middlesex

References

1. Wilson A, Ackers JP. Urine culture for the detection of *Trichomonas vaginalis* in men. *Br J Vener Dis* 1980;**56**:46-8.
2. Rosedale N. Trichomonal vaginitis is not a venereal disease. *World Medicine* 1977;**12**:33.

TO THE EDITOR, *British Journal of Venereal Diseases*

Treatment of lymphogranuloma venereum with rifampicin

Sir,

With reference to the letter on the "Treatment of lymphogranuloma venereum with rifampicin" by Menke and co-workers,[1] I would like to make some comments on the reasons listed at the end of the letter, on which the authors base their conclusions not to recommend rifampicin for the treatment of lymphogranuloma venereum (LGV) despite the very encouraging results obtained.

The first reason seems to be concerned mainly with safety and is expressed in such a way as to imply indirectly that rifampicin is not safe. This is contradicted by extensive clinical evidence, including the lack of side effects reported by the authors themselves.

It should be pointed out in this context that the administration of rifampicin for relatively long periods in patients with a severe disease, such as tuberculosis, in combination with drugs such as streptomycin and isoniazid and more recently pyrazinamide is associated with an extremely low incidence of side effects. This evidence is based on data collected all over the world on hundreds of thousands of patients. A very exhaustive review of this argument has recently been made by Girling[2] of the British Medical Research Council. (I wonder what the data on the safety of tetracycline would be, had it been given—as was the case for rifampicin in the early phase of use—for periods up to 24 months on a continuous daily basis in the treatment of tuberculosis.)

The second reason refers to an alleged risk of increased resistance of *Mycobacterium tuberculosis* to rifampicin as a result of the use of this antibiotic in non-tuberculous infections. On this particular point, data from various countries where rifampicin is used for both tuberculous and non-tuberculous infections show that the sensitivity of *M tuberculosis* to rifampicin is practically 100% and very constant.[3] In some of these countries, the amount of antibiotic used in non-tuberculous infections (which is a good estimate of the selective pressure exerted on the sensitive bacterial population) is much higher than that used in tuberculosis.

As far as the third reason is concerned, one would expect that a definite clinical recommendation should be based on clinical data and not extrapolated from in-vitro data (which are valid as long as clinically confirmed).

In conclusion I think that the effective therapeutic value of rifampicin in LGV should be more appropriately established through controlled clinical trials and not by statements unsupported by adequate experimental evidence. Furthermore, since the negative attitude is based on preconceived arguments, none of which would have been modified by the results of the study, I wonder why the study was undertaken and published.

Yours faithfully,

G Acocella

European Area Medical Department,
Gruppo Lepetit,
Milan,
Italy

References

1. Menke HE, Schuller JL, Stolz E, Niemel PLA, Michel MF. *Br J Vener Dis* 1979: 55:379.
2. Girling D. *J Antimicrob Chemother* 1977;3:115.
3. Acocella G, Hamilton-Miller JMT, Brumfitt W. *Lancet* 1977;i:740.

Book Reviews

Tropical Venereology. By O P Arya, A O Osoba, and F J Bennett. 1980. Pp 234. Churchill Livingstone, London and Edinburgh. Price £8.00.

Most books on venereology, or sexually transmitted diseases, have been written by authors whose main experience has been obtained at teaching centres in developed countries. While clinical descriptions are usually excellent, the value of sophisticated laboratory techniques for diagnosis may be emphasised. The practitioner in a tropical country, particularly outside the main centres of medicine, may find such techniques have little relevance to his practice. This book aims to fill the gap left by these previous accounts. It is a formidable task but has been ably undertaken by an eminent trio with clinical, epidemiological, and microbiological experience gained in Africa and in Britain. The usual pathological and clinical descriptions are given and the best diagnostic techniques are described. The differential diagnoses are particularly informative showing the need to consider a wider range of conditions in the tropics. Treatments follow standard patterns but there is emphasis on economical prescribing, a practice which can be applied with advantage all over the world. In addition there are appendices on the management of common conditions in situations where facilities are limited; this advice is practical and sensible in many tropical areas where budgets are limited.

Yet another name is introduced for what is widely called non-specific genital infection—namely, non-specific genital inflammatory disease—and some may grumble at the use of an even clumsier term which does nothing to clear an already complex topic. The subject is carefully covered in 19 pages. It is a reflection of the relative importance of such conditions as candidosis in the tropics that the condition is covered in three pages. I had also anticipated more in the chapter on genital manifestations of some tropical diseases. Furthermore, lymphogranuloma venereum can present as a more dramatic systemic illness, in the Far East at least, than is indicated by the description given.

There are useful accounts on the control of sexually transmitted diseases in the tropics, including advice on setting up a clinical service and appropriate laboratory facilities. There is a valuable section on health education and a more sophisticated account of epidemiological techniques in control. There is also a practical account on training auxiliaries who are responsible for primary health care in many rural tropical areas, where much of the population still lives.

One drawback must be mentioned and that concerns the clinical photographs. There are many black-and-white illustrations but their reproduction is of poor quality—presumably to reduce costs and so help make the book available to more readers. Despite this, I can recommend this book, for it succeeds admirably in its aim. It will be most useful to those working in the tropics. It should be read widely elsewhere for, as has been said before, sexually transmitted diseases travel the world at great speeds borne by air travellers.

R N Thin

Antibiotics and Chemotherapy: Current Topics. Edited by R N Grüneberg, 1980. Pp 219. MTP Press, Lancaster. Price £12.95.

It is difficult for most people to keep abreast of the rapidly changing field of antibiotics and chemotherapy. In this book eight topics have been chosen for review which are of general interest. These include a very lucid exposition of the treatment of infective endocarditis, the vexed question of the prophylactic use of antibiotics, the treatment of anaerobic infections, and the use of combinations of antibiotics, as well as an exhaustive review of the cephalosporins, antibiotic policies, and the treatment of gonorrhoea and non-specific genital infection. Venereologists are fortunate in that most of the conditions they commonly deal with respond well to antibiotics, although they need to be aware of changing patterns of sensitivity. particularly of the gonococcus.

The chapter on gonorrhoea and non-specific genital infection is written from a practical standpoint and gives a good description of current practice with references to the recent literature up to 1978. The problems posed by the emergence of β-lactamase-producing strains of gonococci are discussed rather briefly; only spectinomycin and cefuroxime are mentioned as alternative methods of treating infections with these strains. Recent work in the field of non-specific genital infection is described and due emphasis placed on those infections not due to chlamydia for which, in the absence of defined pathogens, treatment still has to be empirical.

These articles provide good reviews of current ideas in a number of important fields and are all backed up by extensive references. The book is well produced with a good index; it is not unduly expensive by today's standards. It can be recommended as a clearly written description of some important applications of antibiotics and their rational use.

A E Wilkinson

Fortschrifte der Praktischen Dermatologie und Venerologie (Advances in Dermatology and Venereology). Edited by O Braun-Falco and H H Wolff, 1979. Pp 396. Springer Verlag, Berlin. Price DM 98, US$54.90.

This volume is somewhat unbalanced, dealing with venereology in 40 pages out of a total of 385. Sections on gonorrhoea and syphilis are mostly confined to the German literature and the penicillin schedules suggested for late syphilis strike one as complex and fussy and include even penicillin infusions. The recent specific immunoglobulin serological tests may point the way to having tests of activity but some doubts on this score still remain.

It is a paradox that while in all Western European countries the relative number of women with gonorrhoea has gone up—approaching that of men—the ratio in West Germany has remained firmly static with three times as many men as women having been diagnosed as being infected.

The number of patients infected with β-lactamase-producing gonococci has remained very low and no routine test to determine the incidence of β-lactamase producers is at present suggested. Systemic gonorrhoea is very well described and illustrated.

In the section dealing with chlamydia-associated urethritis it is good to see that proper weight has been given to the pioneering work of Lindner, a Viennese ophthalmologist, who laid the foundation to subsequent work in this sphere in amazing detail as early as 1908 and 1910.

The long section on chancroid, which is rare in Germany, might appear out of proportion when it is realised that no mention is being made of such important and common conditions as genital herpes, sexually transmitted hepatitis, Reiter's disease, or specific vaginitis and its complications.

All in all, the section on venereal diseases will have little appeal for venereologists and probably give too little practical detail to be of much value to the dermatologist who also dabbles in venereology.

G W Csonka

Notice

Symposium on trichomoniasis

An international symposium on trichomoniasis, organised by the Commission on Trichomoniasis of the General Council of the Polish Parasitological Society, will take place between 13 and 15 July 1981 in the Medical Academy in Białystok as a satellite meeting to the VIIth International Congress of Protozoology (Warsaw, 5-11 July 1981).

The main topics will be: biology of *Trichomonas vaginalis* Donné, including culture; biochemistry and physiology; immunology and serology; clinical and experimental pathology; epidemiology; diagnosis; and treatment and prophylaxis.

Those interested in attending the meeting are asked to apply by 1 January 1981 to either Professor S Soszka (President of the organising committee), Institute of Gynaecology and Obstetrics, Skłodowska Curie Str 24A, 15-097 Białystok, Poland, or Professor B Honigberg (Vice-president), Department of Zoology, University of Massachusetts, Amhert, Massachusetts 01003, USA, or Professor M Müller (vice president), Rockefeller University, New York, NY 10021, USA.

The papers will be published in English in the periodical *Wiadomości Parazytologiczne* after being read at the symposium.

Correction

In the paper by Hobson *et al* on "Quantitative aspects of chlamydial infection of the cervix" (1980;**56**:156-62) the results in the second paragraph on page 156 should be as follows: the proportion of women with mucopus with chlamydial infection alone was 127/260 (48·8%) and not 127/250 (65%) and that of chlamydia-positive women with gonorrhoea was 83/143 (58%).

Br J Vener Dis 1980;**56:**417-423

Index to Volume 56, 1980

Authors and subjects are indexed together. There is a separate index of articles abstracted and another of authors of articles abstracted.

ACKERS J P. *See* WILSON A, **46**

ACOCELLA G: Treatment of lymphogranuloma venereum with rifampicin (correspondence), **414**

ALAUSA K O, and OSOBA A O: Epidemiology of gonococcal vulvovaginitis among children in the tropics, **239**

AL-EGAILY S S. *See* DUNLOP E M C *et al,* **337**

ALERGANT C D. *See* ARYA O P *et al,* **117**

AL-SOWAYGH I A. *See* HANNA N F *et al,* **227**

Amoebiasis in New York City, **347**

Amoxycillin, effect of probenecid on, **183, 184**

Ampicillin and bacampicillin compared in the treatment of gonorrhoea, **151**

Amylase, induction of cell wall variant of gonococcus by, **230**

ANDERSON A-L. *See* MÅRDH P-A *et al,* **327**

ANNELS E H. *See* ARYA O P *et al,* **117**

ANONG C N. *See* MONTEFIORE D *et al,* **49**

ARYA O P, ALERGANT C D, ANNELS E H, CAREY P B, GHOSH A K, and GODDARD A D: Management of non-specific urethritis in men (correspondence), **117**

Autolymphocytotoxins in syphilis, **297**

Bacampicillin and ampicillin compared in the treatment of gonorrhoea, **151**

BAILEY J C. *See* COLE C H *et al,* **314**

BAIN S S R. *See* McMILLAN A *et al,* **223**

BANFFER J R J. *See* VAN DER LUGT B *et al,* **148**

BARBHAIYA R. *See* TURNER P *et al,* **184**

BARROW J. *See* BLACKWELL A L, **105**

BERGSTEIN N A M: Treatment of vulvovaginal candidal infection with miconazole-treated tampons, **408**

BIJKERK H: Penicillinase-producing gonococci in the Netherlands, **243**

BIRRY A. *See* KASATIYA S, **77**

BLACKWELL A L and BARROW J: Carcinoma of bladder presenting as gonococcal cystitis, **105**

Bladder carcinoma presenting as gonococcal cystitis, **105**

Blood donors, antichlamydial antibody in London, **404**

BOBECK S. *See* MÅRDH P-A *et al,* **96**

BOER A M. *See* de JONG N H L *et al,* **297**

BOOK REVIEWS

  ARYA O P, OSOBA A O, and BENNETT F J: Tropical Venereology (1980), **415**

  BRAUN-FALCO O and WOLFF H H (editors): Fortschrifte der Praktischen Dermatologie und Venerologie (Advances in Dermatology and Venereology) (1980), **416**

  BARLOW D: Sexually Transmitted Diseases: The Facts (1979), **185**

  CATTERALL R D: Venereology and Genitourinary Medicine 2nd edition (1979), **271**

  GRUNEBERG R N (editor): Antibiotics and Chemotherapy (1980), **416**

  HUFF R W, and PAUERSTEIN C J: Human Reproduction (1979), **271**

  OBER W B: Boswell's Clap and Other Essays: Medical Analyses of Literary Men's Afflictions (1979), **185**

  REDFERN P. The Love Diseases (1979), **271**

  SCHACHTER J and DAWSON C R: Human Chlamydial Infections (1978), **58**

  WILLCOX R R. Management of sexually transmitted diseases: a guide for the general practitioner, **118**

BOROBIO M V, NOGALES M C, and PALOMARES J C: Value of serological diagnosis in congenital syphilis: report of nine cases, **377**

BOS J D, HAMERLINCK F, and CORMANE R H: Antitreponemal IgE in early syphilis, **20**

BOS J D, HAMERLINCK F, and CORMANE R H: Immunoglobulin-bearing lymphoid cells in primary syphilis, **69**

BOS J D, HAMERLINCK F, and CORMANE R H: Immunoglobulin-bearing polymorphonuclear leucocytes in primary syphilis, **218**

BOS J D, HAMERLINCK F, and CORMANE R H: T-lymphoid cells in primary syphilis, **74**

BOWIE W R, YU J S, FAWCETT A, and JONES H D: Tetracycline in nongonococcal urethritis: comparison of 2 g and 1 g daily for seven days, **332**

BRAUN D W. *See* GENVERT *et al,* **400**

BREWERTON D A. *See* DAROUGAR S *et al,* **404**

BRITISH CO-OPERATIVE CLINICAL GROUP: Homosexuality and venereal disease in the United Kingdom, **6**

BRUUN L. *See* FENNESTAD K L *et al,* **198**

BYNG R E. *See* HOBSON D *et al,* **156**

BYNG R E. *See* TAIT I A *et al,* **37**

Candida, vulvovaginal, treatment with miconazole tampons, **408**

CAREY P B and JOHNSON J N: Diverticular disease presenting at a special clinic (correspondence), **57, 348**

CAREY P B. *See* ARYA O P *et al,* **117**

CATTERALL R D: Confidentiality in the National Health Service and in the service for sexually transmitted diseases, **263**

CATTERALL R D: First African Regional Conference on Sexually Transmitted Diseases, **115**

CATTERALL R D: International Union against the Venereal Diseases and Treponematoses, **1**

CATTERALL R D: Sexually transmitted diseases in Nigeria, **194**

Cefuroxime, treatment of gonococci in Rotterdam with, **249**

Cephalosporin, HR 756, in treatment of *N gonorrhoeae* strains, **255**

Cervix, quantitative aspects of chlamydial infection of, **156**

CHAPEL T A. *See* TAKEGUCHI M M *et al*, **304**
CHIEF MEDICAL OFFICER: Sexually transmitted diseases (extract from annual report for 1978), **178**
Chlamydia, anti-chlamydial antibody in London blood donors, **404**
Chlamydia, contact tracing in patients with genital infection, **259**
Chlamydia trachomatis, cause of FitzHugh-Curtis syndrome?, **341**
C trachomatis colonisation of pregnant and puerperal women and neonates, **96**
C trachomatis, epidemiology of serotypes D to K infection, **163**
C trachomatis in perianal lesion of lymphogranuloma venereum of 20 years' duration, **344**
C trachomatis, prevalence in Greenland by seroepidemiological study, **327**
C trachomatis, subclinical pneumonia, **337**
Chlamydial cervical infections, value of screening, **92**
Chlamydial culture service, **183**
Chlamydial infection of cervix in contacts of men with nongonococcal urethritis, **37**
Chlamydial infection of cervix, quantitative aspects of, **156**
COLE C H, LACHER T G, BAILEY J C, and FAIRCLOUGH D L: Vaginal chemoprophylaxis in the reduction of reinfection in women with gonorrhoea: clinical evaluation of the effectiveness of a vaginal contraceptive, **314**
COLLART P, POITEVIN M, MILOVANOVIC A, HERLIN A, and DUREL J: Kinetic study of serum penicillin concentrations after single doses of benzathine and benethamine penicillins in young and old people, **355**
Condylomata lata of the palms, **267**
Confidentiality in the National Health Service and service for sexually transmitted diseases, **263**
Conjunctivitis due to *H ducreyi*, **414**
COPLEY C G. *See* SHANNON R *et al*, **372**
CORMANE R H. *See* BOS J D *et al*, **20**
CORMANE R H. *See* BOS J D *et al*, **218**
CORMANE R H. *See* BOS J D *et al*, **74**
CORMANE R H. *See* BOS J D *et al*, **69**
CORRESPONDENCE
  ACOCELLA G: Treatment of lymphogranuloma venereum with rifampicin, **414**
  ARYA O P, ALERGANT C D, ANNELS E H, CAREY P B, GHOSH A K, and GODDARD A D: Management of non-specific urethritis in men, **117**
  CAREY P B and JOHNSON J N: Diverticular disease presenting at a special clinic, **57, 348**
  FELMAN Y M and MARR J S: Amoebiasis and giardiasis in New York City: a response, **347**
  GRAVES S, TREWARTHA F, and GOH T: Effect of pH on motility and virulence of *T pallidum* (Nichols) and *T paraluis-cuniculi* in vitro under anaerobic conditions, **269**
  GREGORY J E, HENDERSON R W, and SMITH R: Conjunctivitis due to *Haemophilus ducreyi* infection, **414**
  ROACH M: Piperacillin, **184**
  ROSEDALE N: Trichomonal urethritis in men, **414**
  SCHOFIELD C B S: Future planning of venereological services, **117**
  TAYLOR-ROBINSON D and MUNDAY P E: Chlamydial culture service, **183**
  TURNER P, BARBHAIYA R, and THIN R N: Effect of probenecid on amoxycillin, **184**
  WANG F H: β-lactamase-producing gonococci in Hong Kong, **270**
  WOODCOCK K: Effect of probenecid on amoxycillin, **183**
Co-trimoxazole, efficacy in granuloma venereum, **412**
CSF, microhaemagglutination test in, **77**
CSF, penicillin concentrations in, after different treatments for syphilis, **363**
CSONKA G W. *See* HANNA N F *et al*, **227**
Cystitis, gonococcal, presenting as bladder carcinoma, **105**

DAN M, ROTMENSCH M H, EYLAN E, RUBENSTEIN A, GINSBERG R, and LIRON M: A case of lymphogranuloma venereum of 20 years' duration: isolation of *Chlamydia trachomatis* from perianal lesions, **344**
DAROUGAR S, FORSEY T, BREWERTON D A, and ROGERS K L: Prevalence of antichlamydial antibody in London blood donors, **404**
DAROUGAR S. *See* DUNLOP E M C *et al*, **163, 337**
DAVIES J A. *See* HOBSON D *et al*, **156**
DEHEROGODA P. *See* FLUKER J L *et al*, **397**
DE JONG N H L, KOEHORST J A M, VAN DER SLUIS J J, and BOER A M: Autolymphocytoxins in syphilis, **297**
Dermatological diseases, non-specific positive test results in, **17**
DETAR C C. *See* TAKEGUCHI M M *et al*, **304**
DHARMARATNAM A D. *See* SAROJINI P A *et al*, **302**
DIEZEL W. *See* ENGEL S, **221**
Diverticular disease presenting at a special clinic, **57, 348**
DONALD W H: Assessment of Till-U-Test GC slide, **81**
DRAKE S M, EVANS B A, and GERKEN A: Vaginal pH and microflora related to yeast infections and treatment, **107**
DROGENDIJK A D. *See* VAN DER LUGT B *et al*, **148**
DRUSIN L M. *See* GENVERT G *et al*, **400**
DUNCAN W C. *See* McBRIDE *et al*, **235**
DUNLOP E M C, DAROUGAR S, and TREHARNE J D: Epidemiology of infection by serotypes D to K of *Chlamydia trachomatis*, **163**
DUNLOP E M C, HARRIS R J, DAROUGAR S, TREHARNE J D, and AL-EGAILY S S: Subclinical pneumonia due to serotypes D-K of *Chlamydia trachomatis:* case reports of two infants, **337**
DUREL J. *See* COLLART P *et al*, **355**
DWYER J M. *See* THOMPSON J J *et al*, **210**

Editorial: Gonorrhoea, a continuing problem in the 1980s, **193**
EDMONDS J W. *See* GRAVES S R *et al*, **381**
EDWARDS D I: Mechanisms of selective toxicity of metronidazole and other nitroimidazole drugs, **285**
EDWARDS L D. *See* SPENGLER P J, **151**
EL-RIFAIE M E-S: Condylomata lata of the palms; an unusual site, **267**
ENGEL S and DIEZEL W: Persistent serum immune complexes in syphilis, **221**
EVANS A J, MORRISON G D, and PRICE D J: Prolonged use of Greenland method of treatment of gonorrhoea, **88, 270**

EVANS B A. *See* DRAKE S M *et al*, **107**
EYLAN E. *See* DAN M *et al*, **344**

FAIRCLOUGH D L. *See* COLE C H *et al*, **314**
FAWCETT A. *See* BOWIE W R *et al*, **332**
FELMAN Y M and MARR J S: Amoebiasis and giardiasis in New York City: a response (correspondence), **347**
FENNESTAD K L, BRUUN L, and WEDØ E: Pleural effusion disease agent as passenger of *Treponema pallidum* suspensions from rabbits, **198**
FITZGERALD T J, JOHNSON R C, and WOLFF E T: Sulfhydryl oxidation using procedures and experimental conditions commonly used for *Treponema pallidum*, **129**
FITZGERALD T J and JOHNSON R C: Influence of testicular fluid infected with *Treponema pallidum* on intradermal lesions, **125**
FitzHugh-Curtis syndrome, *C trachomatis*, a possible cause, **341**
FLUKER J L, DEHEROGODA P, PLATT D J, and GERKEN A: Rectal gonorrhoea in male homosexuals: presentation and therapy, **397**
FORSEY T. *See* DAROUGAR S *et al*, **404**
FRASER R K. *See* TELFER-BRUNTON W A *et al*, **325**
FROM E. *See* MÅRDH P-A *et al*, **327**
FROM E: Some aspects of venereal diseases in Greenland, **65**

GANGADHARAN C. *See* SAROJINI P A *et al*, **302**
GARG B R. *See* LAL S, **412**
GEARY I. *See* MELLERSH A *et al*, **230**
General paresis following tabes dorsalis after 20 years, despite penicillin therapy, **368**
Gentamicin treatment for penicillinase-producing gonococci, **394**
GENVERT G, DRUSIN L M, SEYBOLT J F, and BRAUN D W: Evaluations of the Papanicolaou-stained cytological smear as a screening technique for asymptomatic gonorrhoea, **400**
GERKEN A. *See* FLUKER J L *et al*, **397**
GERKEN A. *See* DRAKE S M *et al*, **107**
GHOSH A K. *See* ARYA O P *et al*, **117**
Giardiasis in New York City, **347**
GIBOWSKI M and NEUMANN E: Non-specific positive test results to syphilis in dermatological diseases, **17**
GILES A J H: Tabes dorsalis progressing to general paresis after 20 years despite routine penicillin therapy, **368**
GINSBERG R. *See* DAN M *et al*, **344**
GODDARD A D. *See* ARYA O P *et al*, **117**
GOH T. *See* GRAVES S *et al*, **269**
GOLDMEIER D: Proctitis and herpes simplex virus in homosexual men, **111**
Gonococcal arthritis, an unusual case, **35**
Gonococcal vulvovaginitis among children in tropics, **239**
Gonococci, antibiotic sensitivities and results of treatment with cefuroxime in Rotterdam, **249**
Gonococci, induction of cell wall variants by human amylase, **230**
Gonococci, penicillinase-producing in Hong Kong, **270**
Gonococci, penicillinase-producing in Netherlands, **243**
Gonococci, penicillinase-producing in Rotterdam, **244**
Gonococci, penicillinase-producing, treatment with gentamicin, **394**
Gonococci, sensitivity to penicillin in different racial groups, **308**
Gonococci, transport and isolation methods, an assessment, **390**
Gonorrhoea, a continuing problem in the 1980s, **193**
Gonorrhoea, ano-rectal, in women, diagnosis and management, **319**
Gonorrhoea, asymptomatic, screening for by Papanicolaou cytology smear, **400**
Gonorrhoea, cervical, in women with unwanted pregnancies, **148**
Gonorrhoea, comparative study of ampicillin and bacampicillin, **151**
Gonorrhoea, Greenland method of treatment in, **88**, **270**
Gonorrhoea, rectal, in male homosexuals, **397**
Gonorrhoea, recurrent, relationship between interval between infections and similarity of gonococcal strains in, **31**
Gonorrhoea, vaginal chemoprophylaxis in women against, **314**
Granuloma venereum, efficacy of co-trimoxazole in, **412**
GRAVES S R, EDMONDS J W, and SHEPHERD R C H: Lack of serological evidence for venereal spirochaetosis in wild Victorian rabbits and the susceptibility of laboratory rabbits to *Treponema paraluis-cuniculi*, **381**
GRAVES S, GOH T, and TREWARTHA F: Effect of pH on the motility and virulence of *Treponema pallidum* (Nichols) and *Treponema paraluis-cuniculi* in vitro under anaerobic conditions (correspondence), **269**
GRAVES S: Susceptibility of rabbits venereally infected with *Treponema paraluis-cuniculi* to superinfections with *Treponema pallidum*, **387**
GREGORY J E, HENDERSON R W, and SMITH R: Conjunctivitis due to *Hæmophilus ducreyi* infection (correspondence), **414**
Greenland method of treatment in gonorrhoea, **88**, **270**
Greenland, seroepidemiological study of *N gonorrhoeae* and *C trachomatis*, **327**
Greenland, VD in, **65**
GRUNDER K and PETZOLDT D: Sensitivity of *Neisseria gonorrhoeae* to spectinomycin and thiamphenicol, **252**

Haemophilus ducreyi conjunctivitis, **414**
HAFIZ S. *See* MELLERSH A *et al*, **230**
HAMERLINCK F. *See* BOS J D *et al*, **20**, **69**, **74**, **218**
HANNA N F, TAYLOR-ROBINSON D, CSONKA G W, HARRIS J R W, and AL-SOWAYGH I A: One-step staining of *Neisseria gonorrhoeae* in urethral discharge by methyl green-pyronin, **227**
HARAHAP M: Sexually transmitted diseases in Indonesia, **282**
HARRIS A B. *See* YOUNG H *et al*, **322**
HARRIS J R W. *See* HANNA N F *et al*, **227**
HARRIS R J. *See* DUNLOP E M C *et al*, **337**
HEBRANT F. *See* VIARENGO J *et al*, **169**
HELIN I. *See* MÅRDH P-A *et al*, **96**
HENDERSON R W. *See* GREGORY J E *et al*, **414**
HERLIN A. *See* COLLART P *et al*, **355**
Herpes simplex virus and proctitis in homosexuals, **111**
Herpes simplex virus type 2 infection in Ibadan, **49**
HOBSON D, KARAYIANNIS P, BYNG R E, REES E, TAIT I A, and DAVIES J A: Quantitative aspects of chlamydial infection of the cervix, **156**
HOBSON D. *See* TAIT I A *et al*, **37**

Homosexuals, proctitis and herpes simplex virus in, **111**
Homosexuals, rectal gonorrhoea in, **397**
Homosexuality and venereal disease in the United Kingdom, **6**
Hong Kong, β-lactamase-producing gonococci in, **270**
HORN F. *See* LUGER A *et al*, **12**
HR 756 in treatment of *N gonorrhoeae* strains, **255**

Ibadan, herpes simplex virus type 2 infection in, **49**
IgA, antigonococcal, detection in cervical secretions by indirect immunofluorescence, **223**
IgE, antitreponemal, in early syphilis, **20**
Indonesia, STD in, **282**
International Union against the Venereal Diseases and Treponematoses, **1**

JOHNSON J N. *See* CAREY P B, **57, 348**
JOHNSON R C. *See* FITZGERALD T J, **125, 129**
JONES H D. *See* BOWIE W R *et al*, **332**

KANERVA L. *See* PAAVONEN J *et al*, **101**
KARAYIANNIS P. *See* HOBSON D *et al*, **156**
KASATIYA S and BIRRY A: Further evaluation of microhaemagglutination test to determine treponemal antibodies in CSF, **77**
KING D M. *See* RODIN P *et al*, **308**
KNOX J M. *See* McBRIDE M E *et al*, **235**
KOEHORST J A M. *See* de JONG N H L *et al*, **297**
KOUSA M. *See* PAAVONEN J *et al*, **101**
KOZAKIEWICZ J. *See* WRZOLKOWA T, **137**

LACHER T G. *See* COLE C H *et al*, **314**
Lagos, sexually transmitted diseases in clinic patients in, **54**
LAL S and GARG B R: Further evidence of the efficacy of co-trimoxazole in granuloma venereum, **412**
LASSUS A. *See* PAAVONEN J *et al*, **101**
LAURIN J. *See* MÅRDH P-A *et al*, **96**
LEE R. *See* THOMPSON J J *et al*, **210**
LIM A L. *See* SNG E H *et al*, **311**
LIND I. *See* MÅRDH P-A *et al*, **327**
LIRON M. *See* DAN M *et al*, **344**
LIVSEY K A. *See* TAKEGUCHI M M *et al*, **304**
London, anti-chlamydial antibody in blood donors in, **404**
LUGER A, SCHMIDT B, SPENDLINGWIMMER I, and HORN F: Recent observations on the serology of syphilis, **12**
Lymphogranuloma venereum of 20 years' duration: *C trachomatis* in perianal lesions, **344**
Lymphogranuloma venereum, treatment with rifampicin, **414**

McBRIDE M E, DUNCAN W C, and KNOX J M: Bacterial interference of *N gonorrhoeae* by α-haemolytic streptococci, **235**
McDONALD H C. *See* TAKEGUCHI M M *et al*, **304**
McENTEGART M G. *See* MELLERSH A *et al*, **230**
McMILLAN A, McNEILLAGE G, YOUNG H, and BAIN S S R: Detection of antigonococcal IgA in cervical secretions by indirect immunofluorescence: an evaluation as a diagnostic test, **223**
McNEILLAGE G. *See* McMILLAN A *et al*, **223**
MANGI R J. *See* THOMPSON J J *et al*, **210**
MÅRDH P-A, HELIN I, BOBECK S, LAURIN J, and NILSSON T: Colonisation of pregnant and puerperal women and neonates with *C trachomatis*, **96**
MÅRDH P-A, LIND I, FROM E, and ANDERSON A-L: Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in Greenland: a seroepidemiological study, **327**
MÅRDH P-A. *See* THELIN I *et al*, **259**
MARR J S. *See* FELMAN Y M, **347**
MELLERSH A, HAFIZ S, McENTEGART M G, and GEARY I: Induction of cell wall variant of the gonococcus by human amylase, **230**
Methyl green-pyronin staining of *N gonorrhoeae*, **227**
Metronidazole, mechanisms of selective toxicity, **285**
MICHEL M F. *See* NAYYAR K C *et al*, **244, 249**
MICHEL M F. *See* NOBLE R C *et al*, **31**
Miconazole tampons as treatment for vulvovaginal candidosis, **408**
Microhaemagglutination test in CSF, **77**
MILLER B R. *See* NOBLE R C, **26**
MILOVANOVIC A. *See* COLLART P *et al*, **355**
MONTEFIORE D, SOGBETUN A O, and ANONG C N: Herpes virus hominis type 2 infection in Ibadan, **49**
MORRISON G D. *See* EVANS A J *et al*, **88, 270**
MORRISON G D. *See* SHANNON R *et al*, **372**
MSSVD annual report, **182**
MUNDAY P E. *See* TAYLOR-ROBINSON D, **183**

NAYYAR K C, MICHEL M F, and STOLZ E: Antibiotic sensitivities of gonococci isolated in Rotterdam and results of treatment with cefuroxime, **249**
NAYYAR K C, NOBLE R C, MICHEL M F, and STOLZ E: Gonorrhoea in Rotterdam caused by penicillinase-producing gonococci, **244**
Neisseria gonorrhoeae, auxotypes and antimicrobial susceptibilities in black and white patients, **26**
N gonorrhoeae, bacterial interference of, by α-haemolytic streptococci, **235**
N gonorrhoeae, characterisation of, from women with simultaneous infections at two sites, **3**
N gonorrhoeae, delay and inhibition of growth in vitro, **83**
N gonorrhoeae, enzymatic detection of, **304**
N gonorrhoeae, identification of, in the routine laboratory, **144**
N gonorrhoeae, penicillinase-producing and ordinary, treatment by HR 756, **255**
N gonorrhoeae, penicillinase-producing, comparison of methods for detection of, **311**
N gonorrhoeae, prevalence in Greenland by seroepidemiological study, **327**
N gonorrhoeae, sensitivity to spectinomycin and thiamphenicol, **252**
N gonorrhoeae, staining with methyl green-pyronin, **227**
N lactamica, isolation from the female genital tract, **325**
Neisserial infection, individual susceptibility to and oropharyngeal flora, **322**
Netherlands, penicillinase-producing gonococci in, **243**
NEUMANN E. *See* GIBOWSKI M, **17**
New York, amoebiasis and giardiasis in, **347**
Nigeria, STD in, **194**
NILSSON T. *See* MÅRDH P-A *et al*, **96**

NOBLE R C and MILLER B R: Auxotypes and antimicrobial susceptibilities of *Neisseria gonorrhoeae* in black and white patients, **26**
NOBLE R C: Characterisation of *Neisseria gonorrhoeae* from women with simultaneous infections at two sites, **3**
NOBLE R C, ORANJE A P, MICHEL M F, and STOLZ E: Relationship of interval between infections and the similarity of gonococcal strains in recurrent gonorrhoea, **31**
NOBLE R C. *See* NAYYAR K C *et al*, **244**
NOGALES M C. *See* BOROBIO M V *et al*, **377**
Nongonococcal urethritis, chlamydial infection of cervix in contacts of men with, **37**
Nongonococcal urethritis, comparison of tetracycline 1 g and 2 g daily for seven days, **332**
Non-specific urethritis, management in men, **117**
North Pacific, VD in islands of, **173**

OBITUARIES
  MARSHALL J, **349**
  QUERESHI S H, **349**
ORANJE A P. *See* NOBLE R C *et al*, **31**
Oropharyngeal flora and individual susceptibility to neisserial infection, **322**
OSOBA A O. *See* ALAUSA K O, **239**

PAAVONEN J and VALTONEN V V: *Chlamydia trachomatis* as a possible cause of peritonitis and perihepatitis in a young woman, **341**
PAAVONEN J, KOUSA M, SAIKKU P A, VARTIAINEN E, KANERVA L, and LASSUS A: Treatment of nongonococcal urethritis with trimethoprim-sulphadiazine and with placebo, **101**
Pacific, South, VD in islands of, **204**
Palms, condylomata lata of, **267**
PALOMARES J C. *See* BOROBIO M V *et al*, **377**
PANG R. *See* RAJAN V S *et al*, **255**
PANG R. *See* RAJAN V S *et al*, **394**
Papanicolaou cytology smear, use for screening asymptomatic gonorrhoea, **400**
Papua New Guinea, VD in, **277**
PAUL I D. *See* RICHMOND S J *et al*, **92**
PAVITHRAN K. *See* SAROJINI P A *et al*, **302**
Penicillin concentrations in CSF after different treatments for syphilis, **363**
Penicillin sensitivity of gonococci in different racial groups, **308**
Penicillins, benzathine and benethamine, kinetic study of serum concentrations, **355**
PETZOLDT D. *See* GRUNDER K, **252**
pH, effect of, on motility and virulence of *T pallidum* and *T paraluis-cuniculi*, **269**
PHILLIPS I. *See* TAYLOR E, **390**
PIOT P. *See* VIARENGO J *et al*, **169**
Piperacillin, **184**
PLATT D J. *See* FLUKER J L *et al*, **397**
Pleural effusion disease agent as passenger of *T pallidum* suspensions, **198**
Pneumonia, subclinical due to *C trachomatis*, **337**
POITEVIN M. *See* COLLART P *et al*, **355**
POLNIKORN N, WITOONPANICH R, VORACHIT M, VEJJAJIVA S, and VEJJAJIVA A: Penicillin concentrations in cerebrospinal fluid after different treatment regimens for syphilis, **363**

PRICE D J. *See* EVANS A J *et al*, **88, 270**
Probenecid, effect of, on amoxycillin, **183, 184**
Proctitis and herpes simplex virus in homosexuals, **111**
Project Icarus, **270**

RAJAN V S. *See* SNG E H *et al*, **311**
RAJAN V S, SNG E H, PANG R, TAN N J, THIRUMOORTHY T, and YEO K L: HR 756: a new cephalosporin in the treatment of gonorrhoea caused by ordinary and penicillinase-producing strains of *N gonorrhoeae*, **255**
RAJAN V S, TAN N J, PANG R, and SNG E H: Gentamicin in the treatment of infections due to penicillinase-producing gonococci, **394**
REES E. *See* HOBSON D *et al*, **156**
REES E. *See* TAIT I A *et al*, **37**
RENNEY K M: An unusual case of gonococcal arthritis, **35**
RICHMOND S J, PAUL I D, and TAYLOR P K: Value and feasibility of screening women attending STD clinics for cervical chlamydial infections, **92**
Rifampicin treatment of lymphogranuloma venereum, **414**
ROACH M: Piperacillin (correspondence), **184**
ROBERTSON D H H. *See* YOUNG H *et al*, **322**
RODIN P, SETH A D, KING D M, and WILKINSON A E: Sensitivity to penicillin of gonococci in different racial groups, **308**
ROGERS K L. *See* DAROUGAR S *et al*, **404**
ROSEDALE N: Trichomonal urethritis in men (correspondence), **414**
ROTIMI V O and SOMORIN A O: Sexually transmitted diseases in clinic patients in Lagos, **54**
ROTMENSCH M H. *See* DAN M *et al*, **344**
Rotterdam, antibiotic sensitivities and results of treatment with cefuroxime of gonococci in, **249**
Rotterdam, penicillinase-producing gonococci in, **244**
RUBENSTEIN A. *See* DAN M *et al*, **344**
RUFLI T: Identification of *Neisseria gonorrhoeae* in the routine venereological laboratory, **144**

SAIKKU P A. *See* PAAVONEN J *et al*, **101**
SAROJINI P A, DHARMARATNAM A D, PAVITHRAN K, and GANGADHARAN C: Concentric rings simulating tinea imbricata in secondary syphilis, **302**
SCHMIDT B. *See* LUGER A *et al*, **12**
SCHOFIELD C B S: Future planning of venereological services (correspondence), **117**
SETH A D. *See* RODIN P *et al*, **308**
SEYBOLT J F. *See* GENVERT G *et al*, **400**
SHANNON R, COPLEY C G, and MORRISON G D: Immunological responses in late syphilis, **372**
SHEPHERD R C H. *See* GRAVES G R *et al*, **381**
SMITH D K. *See* TAKEGUCHI M M *et al*, **304**
SMITH R. *See* GREGORY J E *et al*, **414**
SNG E H. *See* RAJAN V S *et al*, **255**
SNG E H. *See* RAJAN V S *et al*, **394**
SNG E H, YEO K L, RAJAN V S, and LIM A L: Comparison of methods for the detection of penicillinase-producing *N gonorrhoeae*, **311**
SOGBETUN A O. *See* MONTEFIORE D *et al*, **49**
SOMORIN A O. *See* ROTIMI V O, **54**
Spectinomycin, sensitivity of *N gonorrhoeae* to, **252**
SPENDLINGWIMMER I. *See* LUGER A *et al*, **12**

SPENGLER P J and EDWARDS L D: Comparative study of bacampicillin and ampicillin in the treatment of uncomplicated gonorrhoea, **151**
STANSFIELD V A: Diagnosis and management of ano-rectal gonorrhoea in women, **319**
STD (extract from Annual Report of CMO for 1978), **178**
STD, First African Regional Conference on, **115**
STD in clinic patients in Lagos, **54**
STD in Indonesia, **282**
STD in Nigeria, **194**
STOLZ E. *See* NAYYAR K C *et al,* **244, 249**
STOLZ E. *See* NOBLE R C *et al,* **31**
Streptococci, α-haemolytic, bacterial interference of *N gonorrhoeae* by, **235**
Syphilis, autolymphocytotoxins in, **297**
Syphilis, congenital, value of serological diagnosis in, **377**
Syphilis, CSF penicillin concentrations after different treatments for, **363**
Syphilis, early, antitreponemal IgE in, **20**
Syphilis, experimental, microhaemagglutination assay for *T pallidum* antibodies, **291**
Syphilis, immunoregulatory properties of serum from patients with different stages of, **210**
Syphilis, late, immunological responses in, **372**
Syphilis, non-specific positive test results in dermatological diseases, **17**
Syphilis, persistent serum immune complexes in, **221**
Syphilis, primary, immunoglobulin-bearing lymphoid cells in, **69**
Syphilis, primary, immunoglobulin-bearing polymorphonuclear leucoctyes in, **218**
Syphilis, primary, T-lymphoid cells in, **74**
Syphilis, primary, ultrastructure of vascular and connective tissue changes in, **137**
Syphilis, secondary, concentric rings simulating tinea imbricata in, **302**
Syphilis serology, lack of evidence for venereal spirochaetosis in wild Victorian rabbits, **381**
Syphilis, serology, recent observations on, **12**

Tabes dorsalis progressing to general paresis after 20 years despite penicillin therapy, **368**
TAIT I A, REES E, HOBSON D, BYNG R E, and TWEEDIE M C K: Chlamydial infection of the cervix in contacts of men with nongonococcal urethritis, **37**
TAIT I A. *See* HOBSON D *et al,* **156**
TAKEGUCHI M M, WEETALL H H, SMITH D K, McDONALD H C, LIVSEY K A, DETAR C C, and CHAPEL T A: Enzymatic detection of *N gonorrhoeae,* **304**
TAN N J. *See* RAJAN V S *et al,* **255**
TAN N J. *See* RAJAN V S *et al,* **394**
TAYLOR E and PHILLIPS I: Assessment of transport and isolation methods for gonococci, **390**
TAYLOR P K. *See* RICHMOND S J *et al,* **92**
TAYLOR-ROBINSON D and MUNDAY P E. Chlamydia culture service (correspondence), **183**
TAYLOR-ROBINSON D. *See* HANNA F *et al,* **227**
TELFER-BRUNTON W A, YOUNG H, and FRASER R K: Isolation of *Neisseria lactamica* from the female genital tract, **325**
Tetracycline, comparison in nongonococcal urethritis of 1 g and 2 g daily for seven days, **332**
THELIN I, WENNSTRÖM A-M, and MARDH P-A: Contact tracing in patients with genital chlamydial infection, **259**
Thiamphenicol, sensitivity of *N gonorrhoeae* to **252**
THIN R N. *See* TURNER P *et al,* **184**
THIRUMOORTHY T. *See* RAJAN V S *et al,* **255**
THOMPSON J J, MANGI R J, LEE R, and DWYER J M: Immunoregulatory properties of serum from patients with different stages of syphilis, **210**
TIGHT R R and WHITE A C: Quantitative microhaemagglutination assay for *Treponema pallidum* antibodies in experimental syphilis, **291**
Till-U-Test GC slide, assessment of, **81**
Tinea imbricata, concentric rings simulating secondary syphilis, **302**
TREHARNE J D. *See* DUNLOP E M C *et al,* **163, 337**
Treponema pallidum antibodies, microhaemagglutination assay for, **291**
T pallidum, effect of pH on, **269**
T pallidum-infected testicular fluid on intradermal lesions, **125**
T pallidum, survival of, in relation to sulfhydryl oxidation, **129**
T pallidum, susceptibility of rabbits with *T paraluis-cuniculi* to superinfections with, **387**
T pallidum suspensions, pleural effusion disease agent as passenger of, **198**
T paraluis-cuniculi, effect of pH on, **269**
T paraluis-cuniculi, rabbits with, susceptibility to superinfections of *T pallidum,* **387**
T paraluis-cuniculi, susceptibility of laboratory rabbits to, **387**
TREWARTHA F. *See* GRAVES S *et al,* **269**
Trichomonas vaginalis, causing male urethritis, **414**
T vaginalis in men, urine culture for detection of, **46**
Trimethoprim-sulphadiazine and placebo in nongonococcal urethritis, **101**
TURNER P, BARBHAIYA R, and THIN R N: Effect of probenecid on amoxycillin (correspondence), **184**
TWEEDIE M C K. *See* TAIT I A *et al,* **37**

United Kingdom, venereal disease and homosexuality in, **6**
Ureaplasma urealyticum in the urethra of healthy men, **169**
Urethritis from *T vaginalis,* **414**
Urethritis, nongonococcal, treatment with trimethoprim-sulphadiazine and placebo, **101**

VALTONEN V V. *See* PAAVONEN J, **341**
VAN DER LUGT B, DROGENDIJK A D, and BANFFER J R J: Prevalence of cervical gonorrhoea in women with unwanted pregnancies, **148**
VAN DER SLUIS J J. *See* DE JONG N H L *et al,* **297**
VARTIAINEN E. *See* PAAVONEN J *et al,* **101**
Venereal disease and homosexuality in the United Kingdom, **6**
VD in Greenland, **65**
VD in islands of North Pacific, **173**
VD in islands of South Pacific, **204**
VD in Papua New Guinea, **277**
Venereological services, future planning of, **117**
VEJJAJIVA A. *See* POLNIKORN N *et al,* **363**
VEJJAJIVA S. *See* POLNIKORN N *et al,* **363**

VIARENGO J, HEBRANT F, and PIOT P: *Ureaplasma urealyticum* in the urethra of healthy men, **169**
VORACHIT M. *See* POLNIKORN N *et al,* **363**
Vulvovaginitis, gonococcal, among children in tropics, **239**

WANG F H: *β*-lactamase-producing gonococci in Hong Kong (correspondence), **270**
WEDØ E. *See* FENNESTAD K L *et al,* **198**
WEETALL H H. *See* TAKEGUCHI M M *et al,* **304**
Wellcome Museum of History of Medicine, **348**
WENNSTRÖM A-M. *See* THELIN I *et al,* **259**
WESTBROOK W G III: Delay and inhibition of growth of *N gonorrhoeae* in vitro, **83**
WHITE A C. *See* TIGHT R R, **291**
WILKINSON A E. *See* RODIN P *et al,* **308**
WILLCOX R R: Venereal diseases in the islands of the North Pacific, **173**
WILLCOX R R: Venereal diseases in the islands of the South Pacific, **204**
WILLCOX R R: Venereal diseases in the Pacific Islands: Papua New Guinea, **277**
WILSON A and ACKERS J P: Urine culture for the detection of *Trichomonas vaginalis* in men, **46**
WITOONPANICH R. *See* POLNIKORN N *et al,* **363**
WOLFF E T. *See* FITZGERALD T J *et al,* **129**
WOODCOCK K: Effect of probenecid on amoxycillin (correspondence), **183**
WRZOLKOWA T and KOZAKIEWICZ J: Ultrastructure of vascular and connective tissue changes in primary syphilis, **137**

Yeast infections, vaginal pH and microflora related to, **107**
YEO K L. *See* RAJAN V S *et al,* **255**
YEO K L *See* SNG E H *et al,* **311**
YOUNG H, HARRIS A B, and ROBERTSON D H H: Oropharyngeal flora and individual susceptibility to neisserial infection, **322**
YOUNG H. *See* McMILLAN A *et al,* **223**
YOUNG H. *See* TELFER-BRUNTON W A *et al,* **325**
YU J S. *See* BOWIE W R *et al,* **332**

Br J Vener Dis 1980;56:424-429

Index to subjects of articles abstracted, 1980

**Indicates that only the title is given.*

Acholeplasma laidlawii, colony structure, ultrastructure and morphogenesis, 275*
Actinomycosis, genital, 64*
Acycloguanosine for systemic herpes, 190*
Acycloguanosine, inhibition of herpes virus, 63*
Acycloguanosine, resistance of herpes simplex virus to, 353*
Amoebiasis in New York 1958-78, identification of homosexuals, 354
Amoxycillin absorption and penetration in pelvic inflammatory disease, 191
Amoxycillin, activity against *C trachomatis* in mice, 62*
Amoxycillin and ampicillin, comparison in treatment of gonorrhoea, 61*
Amphotericin B, phenotypic resistance to, in *C albicans,* 275*
Ampicillin, activity against *C trachomatis* in mice, 62*
Ampicillin and amoxycillin, comparison in treatment of gonorrhoea, 61*
Ankylosing spondylitis and chlamydial infection in HLA-B27 blood donors, 122*
Arthritis and non-gonococcal urethritis, HLA and clinical features, 122
Azlocillin, activity against *N gonorrhoeae* and *H influenzae,* 351*

Behçet's disease and multiple sclerosis, clinical comparative study of, 354*
Behçet's disease, HLA-B5 and pathergy test in, 276*
Behçet's disease, inner ear involvement in, 354*
Behçet's disease, oral ulceration of, acute phase proteins in, 191*
Behçet's disease, significance of mast cells in lesions of, 354*
Behçet's syndrome, colchicine in necrotising vasculitis in, 191*
Behçet's syndrome, pulmonary function in, 276*
β-lactamase inhibitors, 120*
Bladder, neurogenic, in genital herpes, 190*
Bowel disease, inflammatory, chlamydia, cytomegalovirus, and *Yersinia* in, 62*

Candida albicans, abortion associated with intrauterine infection of, 122*
C albicans, adherence to mucosal epithelial cells, 189*
C albicans, alteration of polymorphonuclear leucocyte activity by, 352*
C albicans, autoregulation of germ-tube development, 63*
C albicans, effect of yeast-mycelial transition on yeast growth, 275*
C albicans, germ-tube induction, 275*
C albicans, inhibitors of sterol biosynthesis by imidazole-containing antifungals, 352*
C albicans, mannan from, which activates serum complement, 122*
C albicans, phenotypic resistance to miconazole and amphotericin B, 275*
C albicans, requirement of opsonins for maximal phagocytosis of, 122
Candida antigen, detection in sera by enzyme-linked immunosorbent assay-inhibition technique, 63*
C parapsilosis, identification of, 122*
Candida, sensitivity to yeast killer toxins, 352*
C tropicalis, isolation and characteristation of polyene resistant variant of, 63*
Candidacidal activity of human polymorphs, inhibition by aminoglycosides, 189*
Candidal vulvovaginitis, treatment by overgrowth of lactobacillus, 63*
Candidiasis, efficacy of econazole against, 352*
Carcinoma-in-situ associated with vulval warts, 276
Cefaclor in male gonococcal urethritis, 121*
Cefoperazone, activity against *N gonorrhoeae* and *H influenzae,* 351*
Cefotaxime, activity against *N gonorrhoeae* and *H influenzae,* 351*
Cefotaxime in treatment of β-lactamase-producing *N gonorrhoeae,* 188*
Cefotaxime, use in prepubertal gonococcal vulvovaginitis, 61*
Cefoxitin in penicillinase-producing *N gonorrhoeae,* 120
Cefoxitin in treatment of gonorrhoea, 274*
Cervical atypia, *C trachomatis* infection with, 121*
Cervical cancer, association of herpes simplex virus with, using lymphocyte reactivity, 123*
Cervical chlamydial infection, response to treatment, 121
Chlamydia attachment sites, wheat germ agglutination blockage of, 122*
Chlamydia-group-specific antigen method for preparation of, 189*
Chlamydia in inflammatory bowel disease, 62
Chlamydia trachomatis and Crohn's disease, 274
C trachomatis antibody in cervical secretions as indicator of genital infection, 62
C trachomatis, chlorhexidine against, 189*
C trachomatis, comparison of various McCoy cell treatment procedures, 122
C trachomatis, delayed hypersensitivity, cause of chronic prostatitis?, 121
C trachomatis, drug susceptibility of 15 strains from non-gonococcal urethritis in Italy, 352*
C trachomatis, effect of cortisol on cellular ATP levels, 275*
C trachomatis, effect of cortisol on fructose-1,6-diphosphatase in cells infected with, 352*
C trachomatis, formalised organisms as antigen in micro-immunofluorescence test, 122*

C trachomatis, genital tract to eye infection, tissue culture of, **122***
C trachomatis identification, comparison of Giemsa and immunofluorescence staining, **275***
C trachomatis inclusion formation, suppression by fetal calf serum, **352***
C trachomatis, infection of untreated primary human amnion monolayers with, **189***
C trachomatis infection with cervical atypia, **121***
C trachomatis in mice, activity of amoxycillin, ampicillin and oxytetracycline against, **62***
C trachomatis, in vitro activity of antimicrobials against, **62***
C trachomatis, isolation from lower respiratory tract of adults, **189**
C trachomatis, modulation by cyclic nucleotides and prostaglandins, **275***
C trachomatis, perihepatitis and salpingitis, **274**
C trachomatis, pneumonia of infancy, **274**
C trachomatis pneumonitis, mouse model, **352***
C trachomatis, role in genital tract and associated disease, **189***
C trachomatis salpingitis, mode of spread of infection, **352***
C trachomatis, serology, reticulate bodies as single antigen, **122***
C trachomatis, susceptibility to antibodies in vitro and in vivo, **62***
C trachomatis, techniques for culture and assessing antimicrobial susceptibility of, **189***
C trachomatis type-specific antigen, isolation of, using molecular shift chromatography, **352***
Chlamydial antibodies, immunofluorescence testing for, **122*** *(bis)*
Chlamydial antibodies, preparation of antigens for microimmunofluorescence testing, **121***
Chlamydial endocarditis, **189***
Chlamydial endotoxin-like activity, **122**
Chlamydial genital infection in male infertility, **189***
Chlamydial infection and ankylosing spondylitis in HLA-B27 blood donors, **122***
Chlamydial infection in neonates, a prospective study, **61**
Chlamydial infection of cervix, response to treatment, **121**
Chlamydial infection of mothers and their infants, **61**
Chlamydial urethritis, asymptomatic, and persistent urethral leucocytosis, **188**
Chlorhexidine against *C trachomatis*, **189***
Clindamycin, activity against *H ducreyi*, **275***
Colchicine applications for penile warts, **190***
Colchicine in necrotising vasculitis in Behçet's syndrome, **191***
Concanavalin-A, suppression of lymphocyte response to, by *T pallidum*-infected rabbits, **119***
Co-trimoxazole and trimethoprim, effect of, on development of drug-resistant vaginal and faecal flora, **354***
Co-trimoxazole, resistance of *N gonorrhoeae* to, **351***
Crohn's disease and *C trachomatis*, **274**
CSF, FTA-ABS in neurosyphilis, **119***
CSF, syphilis antibodies in diagnostic significance, **186**
Cyclacillin, potential single-dose treatment for *N gonorrhoeae*, **188***
Cystoid macular oedema in neurosyphilis, **59***
Cytomegalovirus and herpes simplex virus infections compared in low-income women, **123***
Cytomegalovirus in inflammatory bowel disease, **62***

Denver, screening for gonorrhoea and syphilis in homosexual bath-houses, **123***
Donovanosis, primary extragenital disseminated cutaneous, **123***
Doxycycline in mycoplasma infection and infertility, **62**

Ear, inner, involvement in Behçet's disease, **354***
Econazole, efficacy against candidiasis and other vaginal discharges, **352***
Enteric diseases, sexually transmitted, **64***
Epididymitis, acute, prospective study of 50 patients, **63**
Erythromycin, activity against *H ducreyi*, **275***
Erythromycin in treatment of non-gonococcal urethritis, **62**

FitzHugh-Curtis syndrome, **350**
FitzHugh-Curtis syndrome, *C trachomatis* in, **274**
Foetal alcohol syndrome and metronidazole, **63***
FTA-ABS in CSF in neurosyphilis, **119***

Glomerulonephritis, membranous, in congenital syphilis, **119***
Gonococcal antigens, inhibitors in urine of radioimmunoassay of, **61**
Gonococcal antigens in urine, detection by radioimmunoassay, **60**
Gonococcal disseminated infection in mice, **187***
Gonococcal endocarditis, **119**
Gonococcal endocarditis, diagnosis by echocardiography, **60***
Gonococcal infection in infants and children, **60***
Gonococcal ophthalmia neonatorum after caesarian section, **60***
Gonococcal otitis externa, **59***
Gonococcal pelvic inflammatory disease, economics in New York, **119***
Gonococcal plasmids, ecology of, **120***
Gonococcal proctitis with superimposed trauma, **60***
Gonococcal urethritis, comparison of tube and microdilution limulus lysate techniques for diagnosis of, **351**
Gonococcal urethritis, diagnosis by culture of first-voided urine, **60**
Gonococcal urethritis, use of cefaclor in men, **121***
Gonococcal vulvovaginitis in prepubertal girls, **119**
Gonococcal vulvovaginitis, prepubertal, cefotaxime in, **61***
Gonococci and toilet seats, **61**
Gonococci, endocervical, effects of surgical lubricant on, **187***
Gonococci, release of peptidoglycan from growing, **60**
Gonorrhoea, aseptic arthritis after, **272**
Gonorrhoea, comparison of ampicillin and amoxycillin in treatment of, **61***
Gonorrhoea, comparison of two selective media for diagnosis of, **188***
Gonorrhoea, complement-fixation test, **351**
Gonorrhoea, follow-up of patients with pelvic inflammatory disease, **273***
Gonorrhoea in street prostitutes, **64***

Gonorrhoea in women, screening by culture, 120*
Gonorrhoea, prepubertal, spectinomycin in, 352*
Gonorrhoea, rectal, diagnosis and treatment, 274, 274*
Gonorrhoea screening, comparison of three techniques, 188*
Gonorrhoea, screening for, in homosexual bath-houses, 123*
Gonorrhoea, treatment with cefoxitin, 274*

Haemophilus ducreyi, comparison of activity of rosamicin, erythromycin, clindamycin, metronidazole and ornidazole against, 275*
H influenzae, activity of cefoperazine, cefotaxime, moxalactam, azlocillin, mezlocillin, against, 351*
H vaginalis, aminopeptidase activity in, 190*
H vaginalis, and vaginitis, controlled trial of treatment, 190
H vaginalis, comparison of two media for isolation, 64*
Hepatic ultrastructure in secondary syphilis, 119*
Hepatitis A and B, serological markers in chronic liver disease, 276*
Hepatitis A, sexual transmission in homosexuals, 190
Hepatitis B—a reservoir, 64
Hepatitis B and primary carcinoma of liver, 123
Hepatitis B, chronic infection, in homosexuals, 123
Hepatitis B, serological tests in homosexuals, 353
Hepatitis B serology in Greek prostitutes, 353
Hepatitis in homosexuals, 64*
Herpes, genital, colposcopy in early pregnancy, 64
Herpes, genital, transient neurogenic bladder in, 190*
Herpes simplex encephalitis in pregnancy, 190*
Herpes simplex, levamisole plus indomethacin in treatment of, 190*
Herpes simplex virus and cytomegalovirus infections compared in low-income women, 123*
Herpes simplex virus antibodies, immunofluorescence test for, 275
Herpes simplex virus antigen, human T-cell response to, 353*
Herpes simplex virus, association with cervical cancer using lymphocyte reactivity, 123*
Herpes simplex virus, resistance to acycloguanosine, 353*
Herpes simplex virus type 2, exogenous genital reinfection shown by restriction endonuclease finger-printing of DNA, 123*
Herpes simplex virus type 2, neonatal disseminated, immunotherapy and drugs in mouse model, 63*
Herpes simplex virus type 2, storage and transport of cultures, 190*
Herpes simplex virus type 2, subclinical in perinatal period, 123*
Herpes simplex virus type 2, tubular structures in mice cervices infected with, 353*
Herpes simplex virus types 1 and 2, different susceptibilities of rabbits' skins, 190*
Herpetic proctitis and sacral radiomyelopathy in homosexuals, 63*
Herpes virus, inhibition by acycloguanosine, 63*
Herpes, systemic, acyclovir for, 190
HLA and clinical features in non-gonococcal urethritis and reactive arthritis, 122
HLA-B5 in Behçet's disease, 276*
HLA B7 and Peyronie's disease, 64*
HLA-B27 blood donors, ankylosing spondylitis and chlamydial infection in, 122*
HLA-B27 in patients with sero-negative spondarthritides, 122*
HLA-B27-positive brothers, Reiter's syndrome following salmonella infection, 122*
Homosexual bath-houses in Denver and Los Angeles, screening for gonorrhoea and syphilis in, 123*
Homosexual hepatitis, 64*
Homosexuality and syphilis in adolescents, 186
Homosexuals and amoebiasis in New York 1958-78, 354
Homosexuals, chronic hepatitis B infection in, 123
Homosexuals, herpetic proctitis and sacral radiomyelopathy, 63*
Homosexuals, intestinal parasites in, 190
Homosexuals, rectal gonorrhoea in, 274, 274*
Homosexuals, serological tests for hepatitis B, in, 353
Homosexuals, sexual transmission of hepatitis A in, 190
Hypoxanthine requirement of *N gonorrhoeae,* 273*

Immunofluorescence testing for chlamydial antibodies, 121*, 122* *(quater)*
Indomethacin plus levamisole in treatment of herpes simplex, 190*
Infertility and mycoplasma infection, effects of doxycycline, 62
Infertility, male, chlamydial genital infection in, 189*
Intestinal parasites in homosexuals, 190
Intrauterine contraceptive device-users, risk of pelvic inflammatory disease in, 191
Italy, drug susceptibility of 15 strains of *C trachomatis* from non-gonococcal urethritis, 352*

Lactobacillus overgrowth in treatment of candidal vulvovaginitis, 63*
Levamisole plus indomethacin in treatment of herpes simplex, 190*
Liver carcinoma and hepatitis B, 123
Liver disease, chronic, serological markers of hepatitis A and B in, 276*
Los Angeles, screening for gonorrhoea and syphilis in homosexual bath-houses, 123*
Lymecycline in treatment of non-gonococcal urethritis, 62

McCoy cell treatment procedures, comparison of, 122*
Menopausal pre- and post-, comparative study of genital bacteriology in women, 124
Metronidazole, activity against *H ducreyi,* 275*
Metronidazole and the foetal alcohol syndrome, 63*
Metronidazole for non-specific vaginitis, 354*
Metronidazole, lack of evidence for cancer due to, 122*
Metronidazole, mutagenicity of, 63
Metronidazole, no cytogenic effect of, 353*
Metronidazole penetration into rabbits' preformed cavities, 353*
Metronidazole, sister chromatid exchanges in lymphocytes treated with, 275
Mezlocillin, activity against *N gonorrhoeae* and *H influenzae,* 351*
Miconazole, phenotypic resistance to, in *C albicans,* 275*

Molluscum contagiosum, virus-specific and anticellular antibodies in, 123*

Moxalactam, activity against _N gonorrhoeae_ and _H influenzae_, 351*

Multiple sclerosis and Behçet's disease, clinical comparative study of, 354*

Mycoplasma hominis, colony structure, ultrastructure and morphogenesis, 275*

M hominis, different routes of infection of female Grivet monkeys using, 189*

M hominis, orogenital contact and pharyngeal isolation of, 61*

Mycoplasma infection and infertility, effects of doxycycline, 62

Mycoplasma infection, study of fetus and placenta, 121

Mycoplasmas, genital, 352*

Neisseria gonorrhoeae, activity of cefoperazone, cefotaxime, moxalactam, azlocillin, mezlocillin against, 351*

N gonorrhoeae, antibodies in urethral exudates, 120*

N gonorrhoeae, antigenic protein complex in outer membrane of, 188*

N gonorrhoeae, attachment of, to HeLa cells by double radiolabelling, 120*

N gonorrhoeae, auxanographic grouping and typing, 60*

N gonorrhoeae, auxogroup and MIC of penicillin, 273*

N gonorrhoeae, bacteraemia associated with complement deficiency, 60

N gonorrhoeae, biological properties of two pilus types, 188*

N gonorrhoeae, β-lactamase-producing, treatment with cefotaxime, 188

N gonorrhoeae, β-lactamase production when cultured with ethidium bromide, 120*

N gonorrhoeae, cefoxitin in penicillinase-producing, 120

N gonorrhoeae, characteristics of defaulters after treatment of, 187*

N gonorrhoeae, chemical method for detection of, 273

N gonorrhoeae, chromosomal gene transfer via conjugation in, 352*

N gonorrhoeae, comparison of penicillin-binding proteins in strains of, 120*

N gonorrhoeae, confirmation of, using Minitek and slide coagglutination techniques, 120*

N gonorrhoeae, conjugal transfer of R plasmids in, 60

N gonorrhoeae, co-transformation of temperature sensitivity and nutritional markers in, 120*

N gonorrhoeae, detection of antibodies on extra-genital surfaces, 273

N gonorrhoeae, DNA repair capacities of, absence of photoreactivation, 188*

N gonorrhoeae, effects of low ampicillin concentrations on, 120*

N gonorrhoeae, evaluation of culture procedure for identification of, 120*

N gonorrhoeae, evaluation of five serological tests for, 120*

N gonorrhoeae, evaluation of Phadebact gonococcus test for, 188*

N gonorrhoeae, genetic basis for colonial variations in, 61*

N gonorrhoeae, genetic exchange mechanisms in, 61*

N gonorrhoeae, growth in continuous culture, 273*

N gonorrhoeae, immunogenicity of ribosomal preparations from, 351*

N gonorrhoeae, increasing resistance to penicillin and co-trimoxazole, 351*

N gonorrhoeae, interactions of lectins with, 351*

N gonorrhoeae, isolates from women with salpingitis, comparison of virulence markers of peritoneal and Fallopian tube isolates, 351*

N gonorrhoeae, killing of, by human polymorph granule extracts, 120*

N gonorrhoeae, membrane microenvironment using electron spin resonance, 61*

N gonorrhoeae, orbital cellulitis from, in enucleated socket, 187

N gonorrhoeae, ornithine transcarbamylase from, 274*

N gonorrhoeae, orogenital contact and pharyngeal isolation of, 61*

N gonorrhoeae, outer membrane proteins of, 351*

N gonorrhoeae, penicillinase-producing, in-vitro susceptibility of, 120

N gonorrhoeae, pharyngeal, 272, 274

N gonorrhoeae, polygenes and modifier genes for tetracycline and penicillin resistance in, 351*

N gonorrhoeae, protection of chick embryo by monospecific antisera, 120*

N gonorrhoeae, protection of chick embryo by non-specific antisera against, 60*

N gonorrhoeae, requirement of hypoxanthine, 273*

N gonorrhoeae, resistance to normal serum, effects using subcutaneous chambers in guinea pigs, 120*

N gonorrhoeae, sensitivity of, to R-type pyocines, 120*

N gonorrhoeae, serology, classification by co-agglutination, 273, 273* _(bis)_

N gonorrhoeae, serotype antigen, analysis by indirect enzyme-linked immunoassay, 351*

N gonorrhoeae, serum bactericidal activity against, 350

N gonorrhoeae, single-dose cyclacillin for, 188*

N gonorrhoeae, slide agglutination confirmation of, 351

N gonorrhoeae, sulphamethoxazole-trimethoprim synergism against, 352*

N gonorrhoeae, type 4, phagocytosis by polymorphs, role of IgG and complement, 188

N gonorrhoeae, virulence associated with surface protein composition, 120*

N gonorrhoeae, wheat germ agglutinin for identification of, 188

N gonorrhoeae, WSJM medium for, 351*

N meningitidis as pathogen in two related cases of epididymitis and pelvic inflammatory disease, 123*

N meningitidis bacteraemia associated with complement deficiency, 60

N meningitidis urethritis, 123

Neisseria, rapid carbohydrate degradation tests for pathogenic, 351

Neurosyphilis, cystoid macular oedema in, 59*

Neurosyphilis, FTA-ABS in CSF, 119*

New York, amoebiasis and identification of homosexuals 1958-78, 354

New York, economics of gonococcal pelvic inflammatory disease in, 119*

Non-gonococcal urethritis and reactive arthritis, HLA and clinical features, 122

Non-gonococcal urethritis, drug susceptibility of 15 strains of *C trachomatis*, 352*
Non-gonococcal urethritis, erythomycin and lymecycline in treatment of, 62

Ornidazole, activity against *H ducreyi*, 275*
Ornithine transcarbamylase from *N gonorrhoeae*, 274*
Osteomyelitis, syphilitic, 59*
Oxytetracycline, activity against *C trachomatis* in mice, 62*

Pachymeningitis cervicalis hypertrophica syphilitica, 186*
Pediculosis pubis, comparison of treatment using synergized pyrethrins and benzene hexachloride, 123
Pediculosis pubis in STD clinic, 276*
Pelvic inflammatory disease, amoxycillin absorption and penetration in, 191
Pelvic inflammatory disease, risk of, amongst IUCD users, 191
Penicillamine, contact reaction of, in vaginal secretions, 64*
Penicillin levels in blood and CSF, 59
Penicillin MIC and auxogroup of *N gonorrhoeae*, 273*
Penicillin resistance in *N gonorrhoeae*, polygenes and modifier genes in, 351*
Penicillin, resistance of *N gonorrhoeae* to, 351*
Peyronie's disease and HLA B7, 64*
Pharynx, orogenital contact and isolation of *N gonorrhoea*, *M hominis*, and *U urealyticum*, 61*
Plasmids, gonococcal, ecology of, 120*
Pneumonia, chlamydial, 274
Pneumonitis, chlamydial, mouse model, 352*
Pregnancy, evaluation of syphilis during, 119*
Prostatism, symptoms, cystometric and urodynamic findings, 191*
Prostatitis, chronic, due to delayed hypersensitivity to *C trachomatis*? 121
Prostitutes and VD 1869-1870, 64*
Prostitutes, gonorrhoea in, 64*
Prostitutes, Greek, hepatitis B serology in, 353
Pulmonary function in Behcet's disease, 276*

Reiter's disease after *S typhimurium* infection, 275*
Reiter's disease in women, 352
Reiter's syndrome, 122*
Reiter's syndrome after salmonella infection in HLA-B27-positive brothers, 122*
Reiter's syndrome, nail involvement in, 189*
Rosamicin, activity against *H ducreyi*, 275*
Rosamicin, activity against *U urealyticum*, 121*
Rosamicin, in-vitro effects on *U urealyticum*, 62*

Salmonella infection before Reiter's syndrome in HLA-B27-positive brothers, 122*
Salmonella typhimurium. Reiter's disease after, 275*
Salpingitis, chlamydial, mode of spread of infection, 352*
Spectinomycin in prepubertal gonorrhoea, 352*
STD, effects of socioeconomic status on incidence of, 124*
STD, social status and sexual behaviour in, 124*
Sulphamethoxazole-trimethoprim synergism against *N gonorrhoeae*, 352*
Syphilis and homosexuality in adolescents, 186
Syphilis antibodies in CSF and their diagnostic significance, 186
Syphilis, congenital, membranous glomerulonephritis in, 119*
Syphilis, congenital neuro-, and juvenile paresis, 272*
Syphilis, early, circulating immune complexes in, 187
Syphilis, endemic, passive transfer of resistance in hamsters, 119
Syphilis, experimental and serology of, in hares, 187*
Syphilis, experimental, passive transfer of immunity with immunoglobulin G, 187*
Syphilis during pregnancy, evaluation of, 119*
Syphilis, infectious, epidemiology of, 119*
Syphilis, late latent, serological responses to treatment of, 272
Syphilis, lymphadenitis in, immunofluorescent identification of spirochaetes from, 186*
Syphilis of rectum mimicking neoplastic disease, 350*
Syphilis, penicillin levels in blood and CSF, 59
Syphilis, screening for, in homosexual bath-houses, 123
Syphilis, secondary, hepatic ultrastructure in, 119*
Syphilis, secondary, histopathology of, 186*
Syphilis serology, comparison of serum and plasma using reagin screen test, 186*
Syphilis serology, use of TPHA test in blood donors, 350*
Syphilis serology, WR, Kline, and VDRL in, 186*
Syphilis, uveitis in, 272
Syphilitic osteomyelitis, 59*

Tetracycline resistance in *N gonorrhoeae*, polygenes and modifier genes in, 351*
Toilet seats and gonococci, 61
Torulopsis, sensitivity to yeast killer toxins, 352*
TPHA test in blood donors, 350*
Treponema pallidum and *T pertenue*, genetic relationship of, 187*
T pallidum, concanavalin A-mediated affinity, film for, 187*
T pallidum, effect on motility of, in acellular culture medium, 119*
T pallidum-infected rabbits, suppression of lymphocyte response to concanavalin-A, 119*
T pallidum-infected testes supernatants in cellular response of normal rabbit lymphocytes, 350
T pallidum, lymphocyte response to, in early experimental syphilis, 186*
T pallidum motility, effect of agents on, in acellular culture medium, 350
T pallidum, rates of clearance from blood of BCG-treated and syphilitic rabbits, 187
T pallidum, receptor-binding proteins and immunogens of virulent, 272*
T pallidum, response of splenic and lymph node lymphocytes to, 187*
T pallidum, solid-phase radio-immunoassay for antibodies to, 59*
T pallidum, surface-associated host proteins on virulent, 186*
T pallidum, testicular cultivation of, facilitated by sustained release steroids, 186*
T pallidum, unique lipoid composition of, 59*
T pertenue and *T pallidum*, genetic relationship of, 187*
Treponemes, electron microscopy of, in TPI test, 59
Trichomonas vaginalis, comparison of five methods for detection of, 353*

T vaginalis, inhibited by oral contraceptives?, **275***
T vaginalis, presentations and laboratory diagnosis of, **353**
T vaginalis, screening by use of acridine orange fluorescence, **275**
T vaginalis, serodiagnosis by indirect fluorescent antibody test, **189**
Trimethoprim, and co-trimoxazole, effect of, on development of drug-resistant vaginal and faecal flora, **354***
Turkey, Behçet's disease in, **276***

Ureaplasma urealyticum, activity of rosamicin against, **121***
U urealyticum, colony structure, ultrastructure and morphogenesis, **275***
U urealyticum, comparison of proteins and polypeptides of serotypes, **275***
U urealyticum, enhancement of growth by putrescine, **189***
U urealyticum growth media, effects of reducing agents and catalase on, **62**
U urealyticum, in-vitro susceptibility to rosamicin, **62***
U urealyticum, modified metabolic inhibition test for serotyping, **62***
U urealyticum, orogenital contact and pharyngeal isolation of, **61***
U urealyticum, survival on different swabs, **121**
Urethra, mucosa of, bacterial colonisation of, **276***
Urethral, peri-, bacterial flora in women, **191***
Uveitis, acquired syphilitic, **272**

Vaginal parasitosis, **191***
Vaginal ulceration, tampon-associated, **191***
Vaginitis, non-specific, metronidazole for, **354***
VD and prostitutes 1869-1870, **64***
Vulva, quantitative microbiology of, **124**

Wart virus infection, non-condylomatous cervical, **354**
Warts, genital, as a sign of sexual abuse in children, **123***
Warts, genital, carcinoma-in-situ associated with, **276**
Warts, penile, treated by topical colchicine, **190***
Warts, vulval, treated by modified simple vulvectomy, **190***
Women with gonorrhoea, screening by culture, **120***

Yeasts, biochemical identification of, **122***
Yersinia in inflammatory bowel disease, **62***

Br J Vener Dis 1980;56:430-434

Index to authors of articles abstracted, 1980

**Indicates that only the title is given.*

Adhar G, 122*
Adinolfi, 191*
Agdal N, 186*
Ahman K, 124
Albritton W L, 275*
Alcalay D, 122*
Alcalay M, 122*
Alderete J F, 186*
Al-Egaily S S, 59
Alexander E R, 63
Alford C A, 123*
Allen P Z, 351*
Alpert S, 61*, 62
Aly R, 124
Amor B, 122*
Anderson I, 120*
Anderson J T, 191*
Andretsch J J, 119*
Andrews B E, 62
Andrews M G, 61
Ansell I D, 276
Apicella M A, 351*
Arango V A, 60*
Arensberg D, 119
Austin T W, 124*

Baker C N, 351*
Baker E H, 120*
Baker H W, 123, 124*
Baker-Zander S A, 186
Ballard R C, 121
Balsdon M J, 190
Bannatyne R M, 119*
Bartenstein L, 273*
Baseman J B, 186*, 187* *(bis)*, 272
Batta R K, 122*
Beard C M, 122*
Bell W M, 275*
Benfari M J F, 60
Bennet D, 123*
Bennet J E, 63*
Bennett R M, 352
Berg R A, 63*
Berg S W, 120
Bergan T, 191
Berger R E, 63
Bergoend H, 187
Berle E J, 353*
Berntsson E, 186*
Bertoloni G, 275*
Bey R F, 119*
Bhargava N C, 123*
Bhargawa S, 122*
Bhujwala R A, 351*
Birch-Andersen, 59
Bittiner J B, 188
Blackshaw A J, 62*
Blackwell C C, 120*
Blair A D, 188*
Block C, 121
Bontoux D, 122*
Borzani E, 350*
Bourlioux P, 190*
Bowser D V, 351*
Brama I, 354*
Bramley M, 275*
Braude A I, 187*
Brause B D, 186*
Briggs J O, 61
Briggs M, 64
Britz M B, 124
Brockman M, 187*
Brooks G F, 60, 351*
Brooks S E H, 119*
Bross D C, 64*
Bucciarelli E, 119*
Buchanan R, 122*
Buchman T G, 123*
Buckell N A, 62*
Burdi M, 354*
Busolo F, 275*

Caldwell H D, 122*
Campbell L A, 188*
Capetanakis J, 186*
Catterall R D, 64
Cevenini R, 352*
Chaffin WL-J, 275*
Chalmers W S K, 119*, 350
Chan J K, 119
Chandra M, 123*
Chang T W, 190*
Chapel T A, 276*
Chen W-J, 352*
Chila M, 351*
Chinn J S, 122*
Choi Y J, 186*
Civatte J, 186*
Clarke S K R, 62
Cleeland R, 62*
Clements M R, 190*
Coleman J C, 64*
Colimon R, 189*
Colleen S, 276*
Combs R S, 189*
Coombs R R A, 60
Connelly M C, 351*
Conventi L, 275*
Cooke D B, 119
Cooper B H, 122*
Cooper M D, 351*
Coppleson M, 354
Coppola S R, 60
Corbeil L B, 187*
Corbeil R R, 187*
Corey L, 190
Corsaro M C, 61*, 123*
Coull I, 120*
Counts G W, 274*
Crockett V A, 62
Cunningham F G, 124*
Cutler J E, 63*
Cwaka W F, 64*
Czaja A J, 276*
Czegledy-Nagy E, 121

Dane D S, 64
Danielsson D, 273, 273* *(bis)*
Danley D L, 352*
Darougar S, 62*, 122*, 275*
Darrow W W, 124*
Davies J, 61* *(bis)*
DeGiusti D, 276*
de Hormaeche R D, 60
De Luvois J, 122*
Dessaint J-P, 187
Devemy P, 187
Digkstra J W E, 189*
Dische M R, 121
Dockerty M B, 122*
Donati M, 352*
Donner A, 61*
Donowho E M, 59*
Doyle R J, 188
Draper D L, 351*
Drogendijk A C, 64*
Drusin L M, 119*
Dublenko S, 120*
Dunlop E M C, 59
Dunn P M, 63*
Dyckman J D, 186*
Dzindzio B S, 60*

Egdell L M, 62
Ehrlich B, 123
Ek S, 272
Elliott W C, 352*
Elmros T, 120*
Eschenbach D A, 124*
Evans B A, 64*
Evans R T, 122*, 352*
Evans W V, 276*
Evrard J R, 62

Faber V, 123
Fahraeus L, 124
Fainaru M, 354*
Falkow S, 60, 120*
Feingold D S, 273
Felman Y M, 60*, 61*, 64*, 123*
Felner J M, 119
Feltham S, 275*
Feng W C, 60
Fenton A C, 120*
Ferchal F, 189*
Ferrante A, 122
Ferrari F A, 189*
Fieldsted A H, 187*
Finch R, 188
Fitzgerald C R, 59*
Fitzgerald T J, 119*
Fiumara N J, 61*, 272, 274
Folds J D, 187*
Forker A D, 60*
Forsey T, 122*, 275*
Fourniat J, 190*
Fouts A C, 353
Frazier J J, 187*
Freundt E A, 189*
Frewen T C, 119*
Friedman G L, 188
Friedmanbirnbaum R, 354*
Friedrich E G, 191*
Frommell G T, 61
Fuchs P C, 61

Gaafar H A, 275
Gale E F, 275*
Garud M A, 191*
Geary I, 120*
German A, 190*
Gilbaugh J H, 61
Gjonnaess H, 191
Good A, 275*
Goodhart G L, 187*
Goldberg I D, 120*, 352*
Goldberg S, 63*
Goldman W D, 354
Goldner M, 120*
Goodner E, 61
Graham D M, 121*. 189*
Graves S, 187
Grayston J T, 122*
Greene L F, 124*
Griffin P S, 123*
Griffiths P D, 62*
Grocholski J, 120*
Grossman M, 61
Grubin L, 124, 188*, 272
Grunberg E, 62*
Grúneberg R N, 124*
Gubish E R, 120*
Guivarc'h M, 350*
Gullberg B, 191

Haak R A, 61*, 188*
Hadler S C, 353
Hafiz S, 120*
Hagdrup H K, 186*
Hagedorn H J, 186
Hagenfeldt K, 124*
Hahn W D, 275*
Haider F, 122*
Haim S, 354*
Haitas B, 121
Hammel I, 354*
Hampton K D, 120*
Hanchard B, 119*
Hann W D, 352*
Hansbrough J, 60*
Hanson A, 122*
Hanto D, 189
Harnisch J P, 63
Harris A B, 120*
Harris R E, 119*
Harrison H R, 189*
Harrison W O, 120
Hartley-Asp B, 63
Hautala J M, 274
Hayes E C, 272*
Hayes N S, 187*
Hazen K C, 63*
Hazen P G, 191*
Hechandex-Perez E, 190*
Heckels J E, 120*
Hellgren L, 120*
Hendry A T, 60*, 273*
Henrichsen C, 351
Hentzer B, 123
Hermans J M H, 352*
Herrin J R, 274
Hildebrandt J F, 120*
Hilger A E, 352*
Hill A C, 121*
Hills E, 354
Hino Y, 353*
Hinthorn D R, 119*
Hinton R A, 62
Hipp S S, 275
Hirschman S Z, 59*
Hochman E B, 274
Hofstad T, 353*
Holm S, 120*
Holmes K K, 63, 124*, 188*, 190, 274*
Holmqvist N D, 123*
Holt J, 61
Hopeman A, 60*
Horne H W, 121
Horton H B, 123, 124*
Horvath I, 187*
Houang E T, 59
Hourihan J T, 352*
Hovind-Hougen K, 59
Hoybye G, 123
Hughes M D, 60
Hunter J, 121
Hunter J C, 276
Hurley R, 122*
Hymans D M, 354*
Isarangkul W, 354
Ito J I, 187*
Iwasaka T, 353*

Jaffe L R, 186
James D G, 124*
James J F, 351*
James L T, 120*
Jenkins H M, 59*
Jenkins R M, 276*
Jindal K, 122*
Johannisson G, 62*, 120*
Johnson A M, 275*
Johnson A P, 275*
Johnson R G, 119*
Johnston K H, 351* *(ter)*
Jones J E, 119*
Jones M B, 122*
Jones R B, 274*
Jones R N, 351*
Judson F N, 123*

Kaklamani E, 353
Kaminski D, 350*
Kaplan M, 123*
Karalis D, 353
Kasper D L, 350
Katta T, 276*
Keat A C, 122
Keller K F, 188
Kelsey M C, 124*
Kemenes F, 187*
Kennell W L, 353*
Kenny G E, 62*, 275*
Kerridge D, 275*
Khokhawalla J, 191*
Kilpatrick M E, 120
King R D, 189*
King R L, 190*
Kingham J G C, 62*
Kinghorn G, 275*
Kirkwood M W, 275
Kjellberg E, 120*
Klein S W, 123*
Kleinhans F W, 61*
Klúge A, 350*
Knox G E, 123*
Kommandaki I, 353
Koornhof J H, 121
Kousa M, 62
Kramer M, 187*
Kramer M J, 62*
Kraus S J, 188, 353
Kreutzer E, 60*
Kundsin R B, 121
Kunin C M, 191*
Kunz L J, 60
Kurland L T, 122*
Kuroiwa Y, 354*
Kusmiesz H, 352*
Kuszmar T, 276*
Kuo C-C, 122* *(bis)*, 352*
Kysiakidou A, 353

Lambden P R, 120*, 188*
Land, G A, 122*
Landini M P, 352*
Laohadtanaphorn S, 276
Larsson P, 186*
Lasala L, 354*
Lassus A, 62
Lato M, 119*
Laughlin C, 61*
Laverty C R, 354
Lebedeff D A, 274
Lee J C, 189*
Lee T J, 60
LeFrock J L, 119
Lehner T, 191*
Leigh B K, 61
Lesser L M, 119
Levett P N, 353*
Levy N J, 122*
Levyzombek E, 119*
Lewis J S, 188*
Lewis V J, 122*
Lichtig C, 354*
Liedholm P, 191
Lim D V, 351*
Lim K S, 64
Linarez L, 120*
Liu C, 119*
Losito A, 119*
Luciano A A, 188*
Lukehart S A, 186*
Lunt P A, 121*
Lycke E, 62*
Lyman D O, 119*
Lynd F T, 353*

Macauley A, 121
McComb D E, 62
McCormack W M, 61*, 62, 350, 352* *(bis)*
McCutchan J A, 120, 187*
McDade R L, 351*
MacDonald A B, 352*
Mace M L, 120*
McEntegart M G, 120*
McGeeney T, 119*
McIntosh K, 61
MacLennan I P B, 63*
McMillan A, 120*, 190, 273, 351
McNeillage G, 120*, 273
Maeland J A, 188*
Maiback H I, 124
Maini R N, 122
Malaviya A N, 122*
Manchee R J, 273*
Manenti G, 354*
Marconi M, 189*
Mårdh P-A, 274 *(bis)*, 276*, 352
Maret S M, 187*
Markham J G, 122*
Marks M I, 63*
Marr J S, 354
Marriott M S, 352*
Martin J E, 188*
Martin N F, 59*
Maskell R, 190
Mason P R, 189
Mason R, 272*
Massibenedetti F, 119*
Matthews H M, 59*
Matthews R S, 123*
Mattioni D, 354*
Mauff A, 352*
Maynard J E, 353
Mead M G, 124*
Medeiros R M, 60
Mehra N K, 122*
Meloni G A, 275*
Merino H I, 123*
Merz W G, 63*
Meurman O, 189*
Meyer B, 121*
Meyer L, 60*
Miao R M, 187*
Michel B, 191*
Middelbeck E J, 352*
Miller J, 187*
Miller J M, 59*
Miller M A, 123*
Millikin P, 123*
Mills J, 61
Minasi P, 190*
Mitchell S H, 122*
Mitelman F, 353*
Moisson-Pulliam T, 187*
Molin L, 124
Møller B R, 189*, 352*
Molnar L, 187*
Moody E E M, 59*
Monda G D, 63
Morgenthau J E, 186
Mori R, 353*
Morris A L, 189*
Morris J E, 190*
Morse S A, 273*
Morton W E, 124*
Motomura S, 354*
Muftnoglu A, 276*
Mulhall A, 122*
Munday P E, 275*
Murphy R L, 353
Murray E S, 60
Muytjens H L, 352*
Myhrberg H, 276*

Nahmias A J, 123*
Nayar M, 123*
Nelson J D, 352*
Neu H C, 354*
Newbold P C H, 64*
Newhall W J, 61*, 188*
Newson J H, 123
Newson S W B, 61*
Ng K M, 121*
Nichols R L, 62
Nielsen H A A, 59
Nikitas J A, 60*
Nikkanen V, 189*
Nisbet I T, 121*, 189*
Noble A D, 122*
Nolan R D, 120*
Noller K L, 122*
Nordling J, 191*
Norlander L, 61* *(bis)*
Normark S, 61* *(bis)*, 120*
Norqvist A, 61*
Novicki D E, 64*

Oates J K, 60, 61*, 63*
Oda H, 353*
Odugbemi T O, 120*
O'Fallon W M, 122*
Oh J O, 190*
O'Leary M, 189
Olhagen B, 272
Olnhausen R W, 122*
Onsrud M, 191
Opitz J L, 124*
Orfila J, 122*
Oriel J D, 62*, 124*
Osborne N G, 124
Osborne W A, 272
Osser S, 191
Osterholm R, 60*
Oxford J S, 63*

Paavonen J, 62, 121*
Pagani A, 189*
Pancoast S J, 354*
Pandhi R X, 351*
Paraskar M, 191*
Pareek S S, 59*
Pass R F, 123*
Paterou M, 186*
Pattison F L M, 124*
Paulsen C A, 63
Pazarli H, 276*
Pead L, 190
Peel R, 63*
Pegrum G D, 122
Penn C W, 120*
Perdiz M, 190*
Perkins R L, 121*
Perol Y, 189*
Petersen B H, 60
Peterson P K, 189
Peutherer J F, 121
Pickering L K, 187*
Pierson D L, 274*
Piette F, 187*
Piot P, 120*
Pizzo P A, 63*
Pizzuto D J, 351
Poirier R H, 353*
Polyak F, 191*
Pomerantz B M, 354

Ponsen H, 123
Post J B, 64*
Postel E, 191*
Potterat J L, 64*
Poulin S A, 121
Powers C N, 274*
Price H L, 62*
Prior R B, 121*, 351
Prober C G, 190*
Pulice R T, 119*
Punnonen R, 189*
Pupontsakis G, 353
Purola E, 121*

Qualls S, 186*
Quinn P A, 121

Racz I, 187*
Ralph E D, 124*
Ram S P, 275*
Rasp F L, 189
Ray L F, 122*
Ray T L, 122*
Reed S I, 275*, 352*
Regan M G, 352
Reid R, 354
Rein M F, 352*
Reiner L, 186*
Rest R F, 120*
Rettig P J, 352*
Reynolds D W, 123*
Reynolds V, 61*
Ricciardi N B, 64*
Rice P A, 350
Richmond S J, 62, 122*
Ridgway G L, 62*
Riggin R T, 189*
Rimland D, 119
Roberts M, 60, 120*
Roberts R B, 186*, 188
Robertson J A, 62*
Robertson J N, 60*, 120*, 188*
Robertson D H H, 120*
Robinson A, 273*
Roelcke D, 350*
Roizman, B, 123*
Ronald A R, 275*
Rosenthal L, 272
Rosenthal R S, 60*, 61*
Rosner B, 62
Ross W H, 272
Rota T R, 189*, 352*
Rothenberg R, 61, 64*
Rubin A, 352*
Ruden A-K, 190*
Rumpianesi F, 352*
Russell J M, 352*
Ryden G, 124

Sabath L D, 189
Sackel S G, 61*
Saikku P, 62, 121*
Saksela E, 121*
Salari H S, 275*
Samarsinghe P L, 63*
Samuel M, 123*
Sandford G R, 63*
Sandgren E, 274
Sandler B, 124*
Sands M, 274*
Sandström E, 273, 273* *(bis)*
San Joaquin V H, 274
Sankaran B, 122*
Sanyal S C, 120*
Saralya V, 191*
Sawyer W D, 61*
Sayed I A, 62*, 275*
Sayer W D, 188*
Schachter J, 61
Schaefer R L, 188
Schaffnit T R, 123*
Schell R F, 119
Schieven B C, 124*
Schiller N L, 188
Schreeder M T, 353
Scott J T, 122
Segal E, 63*
Segura J W, 124*
Sehgal V N, 123*
Seidel J, 123*
Sell S, 186*
Semine D Z, 62
Sernryd A, 62*
Sexton R A, 123*
Sharma N L, 123*
Sheller M J, 353
Shepard M C, 189*
Sheperd G G, 273*
Shepherd M G, 275*
Shibata S A, 120
Shirodaria P V, 123*
Shockley R K, 351*
Shriniwas S, 351*
Siccardi A G, 189*
Siegel D, 59*
Siegesmund K A, 191*
Sigurdson S, 120*
Simmons R L, 189
Simon R, 64
Singh O P, 351*
Singha H S K, 124*
Sjobeg N-O, 191
Skinhoj P, 123
Slack R C B, 188
Smith D L, 352
Smith H, 120*
Smith I W, 121
Smith J, 187*
Smith J W, 123*
Smith K O, 353*
Smith P W, 122*
Smith R F, 64*
Smith T F, 62*
Snyderman R, 60
Soneji A, 63*
Spagna V A, 121*, 351
Spence M R, 187*
Spicer P E, 189*
Stagno S, 123*
Stalder G A, 350*
Stallings R A, 120*
Stavrianeas N, 186*
Stefanoni R, 189*
Steinberg V I, 352*
Steinberg V L, 120*
Steiner S M, 120*
Stemke G W, 62*
Stewart I O, 60*, 273*
Stewart-Brown S, 63*
Stimson J, 274*
Strand C L, 60*
Strombeck B, 353*
Strulovitch C, 63*
Stumm C, 352*
Sturgess J M, 121
Sud I J, 273
Sullivan P A, 275*
Sundal E, 350*
Suni J, 121*
Sutton G, 121*
Sutton H F S, 272
Svarva P L, 188*
Sveen K, 353*
Swarbrick E T, 62*
Swartz S L, 188
Sweet R, 61
Sweet R L, 351*
Sweitzer M, 187*
Swenson J M, 352*
Sworn M K, 122*
Szanto E, 124*
Szeky A, 187*

Tabira T, 354*
Tack K J, 189
Tanzer J, 122*
Taylor G E, 190
Taylor-Robinson D, 119*, 122*, 189*, 275*, 350, 352* *(bis)*
Teare E L, 190*
Tedder R S, 64
Tejani N, 123*
Terho P, 189*
Terry S, 119*
Tewari R P, 351*
Thacker W L, 122*
Thomas B J, 189*, 275*
Thompson D S, 273*
Thompson G R, 275*
Thong Y H, 122
Thornley M J, 60, 61
Thornsberry C, 351*, 352*
Thornton A, 64*
Thorsby E, 353*
Tiamson E M, 64*
Tibbs G J, 189*

Titus R G, 187*
Topfolstein B, 119*
Torres J E, 123*
Toshach S, 120*
Totten, E, 123*
Trent D W, 59*
Trichopoulos D, 353
Tsoutsoplides G C, 190*
Tuzun Y, 276*

Urquhart D, 120*
Ursing B, 274
Ursing W B, 353*

Vaidya M C, 122*
Van Meir J M, 64*
Veale D R, 120*
Vesterinen E, 121*
Vincent J, 120*
Voinchet O, 350*
Volkin L B, 273*
Von-Krogh G, 190*

Wagner K F, 188*
Wall T, 351*
Wang S-P, 61, 122*
Wantzin G L, 186*
Ward M E, 275*
Warner P F, 351*
Washington J A, 351
Wasilauskas B L, 120*
Watt P J, 120*, 188*
Watter S, 191*
Wattre P, 187
Wayman F, 275*
Weiser R S, 187*
Weiss J J, 275*
Wende R D, 186*
Wepper K D, 122*
Weström L, 274
Wiblin C N, 273*
Wicher K, 350*
Wicher V, 350*
Wielenga G, 64*
Wiggelinkhuizen J, 272*
Wilde C E, 188*
William D C, 61*, 123*
Williams R P, 120*
Wills T E, 63*
Willscher M K, 64*
Wilson D V, 60
Winblad B, 120*
Wladmiroff J W, 64*
Wølner-Hanssen P, 274
Woltjen J, 60*
Wong T P, 351*
Wright R C, 124
Wulf H C, 275
Wunderlich A C, 187*

Yank T-K, 59*
Yasbin R E, 188*
Yazici H, 276*
Yeager A S, 190*
Yin C Y, 275*
Yong E C, 122*
Yoshiawa T T, 120
Young H, 120* *(bis)*, 273, 351
Yount F, 119*
Yousuf M, 123*
Yurdakul S, 276*

Zachar Z, 187*
Zaidi A A, 187*
Zinner S H, 62
Zonana J, 123*
Zubrzycki L J, 351*
Zuckerman A J, 64*

FORTY-EIGHT HOUR PATIENCE

One tablet nightly for three nights means that your patient's patience only needs to last 48 hours. That's all it takes to clear vaginal candidiasis – twice as conveniently as the old One Two Free.

Canesten®
clotrimazole

New 200mg vaginal tablet now only one a night for three nights

Presentations Canesten is available either as 200 mg vaginal tablets in packs of three with applicator or as 100 mg vaginal tablets in packs of six with applicator. Canesten Duopak contains six 100 mg vaginal tablets with applicator plus 20 g cream containing 1.0% clotrimazole. **Dosage** *Vaginal Tablets 100mg* Two vaginal tablets should be inserted at night for three consecutive days. Alternatively, one vaginal tablet daily for six days. *Vaginal Tablets 200mg* One vaginal tablet should be inserted at night for three consecutive days. *Duopak* Vaginal Tablets 100 mg as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to prevent re-infection. **Side effects** Rarely patients may experience transient local mild burning or irritation. **Basic NHS Costs** 6 x 100 mg vaginal tablets with applicator £2.13; 3 x 200 mg vaginal tablets with applicator £1.99; Duopak £3.47. **Product Licence Numbers** Vaginal tablets 100 mg, PL 0010/0015; vaginal tablets 200 mg, PL 0010/0072; Duopak, PL 0010/0016 & PL 0010/0015. Further information is available from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. ® Registered Trade Mark of Bayer Germany.

Reduced Subscription Rates for Junior Hospital Doctors Residents and Interns...

British Journal of Venereal Diseases

is available to junior hospital doctors in all parts of the world for a maximum of four consecutive years at REDUCED Annual Subscription Rates. (Thereafter, or when reaching consultant status this special offer will no longer apply.) The following reduced rates are unchanged for the six issues published in 1981.

Inland £13.00
Overseas US$30.00

(The full rates are: Inland £27.00; Overseas US$65.00)

The opportunity to subscribe to this journal of international authority and repute at a substantially reduced subscription rate is one not to be missed by junior doctors seeking to specialise in venereology.

Please complete the adjacent order form and send for your subscription NOW.

Further order forms can be supplied on request.

ORDER FORM

BMA Publications
BMA House, Tavistock Square
London WC1H 9JR, England

Please enter my subscription to

BRITISH JOURNAL OF VENEREAL DISEASES

☐ United Kingdom and Irish Republic £13.00
☐ Overseas US$30.00
☐ My remittance for.................. is enclosed

(Please tick appropriate boxes)

NAME..
....
ADDRESS..................................
....
....

To be completed by Consultant

I hereby confirm that the above mentioned doctor is a member of the junior hospital staff/resident/intern at ....
....
Signed..

BRITISH JOURNAL OF VENEREAL DISEASES

Sexually Transmitted Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $25.00 outside the United States ($22.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $28.00 in the United States and $30.00 in all other countries.

William O. Harrison, M.D.
American Venereal Disease Association
P.O. Box 22349
San Diego, California 92122 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $25.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases.*

☐ I wish to enter a Non-Member or institutional subscription ($28.00 U.S., all other countries $30.00).
(Mail non-member subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105)

Name ______________________________

____

Address ______________________________

City ______________ State Country ______________ Zip ______________

British Journal of Venereal Diseases December 1980 Vol 56 No 6

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Kinetic study of serum penicillin concentrations after single doses of benzathine and benethamine penicillins in young and old people
P COLLART, M POITEVIN, A MILOVANOVIC, A HERLIN, AND J DUREL *page* 355

Penicillin concentrations in cerebrospinal fluid after different treatment regimens for syphilis
NIWAT POLNIKORN, RAWIPHAN WITOONPANICH, MALAI VORACHIT, SODSAI VEJJAJIVA, AND ATHASIT VEJJAJIVA *page* 363

Tabes dorsalis progressing to general paresis after 20 years despite routine penicillin therapy
A J H GILES *page* 368

Immunological responses in late syphilis
R SHANNON, C G COPLEY, AND G D MORRISON *page* 372

Value of serological diagnosis in congenital syphilis: report of nine cases
M V BOROBIO, M C NOGALES, AND J C PALOMARES *page* 377

Lack of serological evidence for venereal spirochaetosis in wild Victorian rabbits and the susceptibility of laboratory rabbits to *Treponema paraluis-cuniculi*
S R GRAVES, J W EDMONDS, AND ROSAMOND C H SHEPHERD *page* 381

Susceptibility of rabbits venereally infected with *Treponema paraluis-cuniculi* to superinfections with *Treponema pallidum*
S GRAVES *page* 387

Assessment of transport and isolation methods for gonococci
E TAYLOR AND I PHILLIPS *page* 390

Gentamicin in the treatment of infections due to penicillinase-producing gonococci
N J TAN, V S RAJAN, ROGER PANG, AND E H SNG *page* 394

Rectal gonorrhoea in male homosexuals: presentation and therapy
J L FLUKER, P DEHEROGODA, D J PLATT, AND A GERKEN *page* 397

Evaluation of the Papanicolaou-stained cytological smear as a screening technique for asymptomatic gonorrhoea
GAIL I GENVERT, LEWIS M DRUSIN, JOHN F SEYBOLT, AND DAVID W BRAUN *page* 400

Prevalence of antichlamydial antibody in London blood donors
S DAROUGAR, T FORSEY, D A BREWERTON, AND K LL ROGERS *page* 404

Treatment of vulvovaginal candidal infection with miconazole-treated tampons
N A M BERGSTEIN *page* 408

Further evidence of the efficacy of co-trimoxazole in granuloma venereum
SARDARI LAL AND B R GARG *page* 412

Correspondence *page* 414

Book reviews *page* 415

Correction *page* 416

Notice *page* 416

Index *page* 417

ASTM CODEN: BJVDAK 56 (6) 355-434 (1980)

ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

16.4.82

February 1982 Vol. 58 No. 1

British Journal of
Venereal Diseases

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

A E WILKINSON
(Editor)
B A EVANS
(Assistant Editor)
A McMILLAN
(Assistant Editor, Abstracts)
J BARROW
(President, MSSVD)

M WAUGH
(Hon Sec, MSSVD)
M W ADLER
G CAUSSE
G CSONKA
L M DRUSIN

J M KNOX
G D MORRISON
I PHILLIPS
E STOLZ
R N THIN
J WALLIN

EDITOR,
British Medical Journal

P D SIMMONS
(Indexer)
SHUNA JUDGE
(Technical Editor)

This *Journal*, founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A McMillan, Royal Infirmary, Dept of Genitourinary Medicine, Lauriston Place, Edinburgh EH3 9YW. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals*, available from the Publishing Manager, *British Medical Journal*, BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; **55**: 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS, Rodin P. *Venereal Diseases*. 4th ed. London: Baillière and Tindall, 1980.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977: 214-22.

PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £31.00 in the United Kingdom and Republic of Ireland and US$76.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US$76.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal*, 1172 Commonwealth Avenue, Boston, Mass. 02134. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases*, BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1982 by the *British Journal of Venereal Diseases*. This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.

16.9.82

NEW

FLAGYL-S

benzoylmetronidazole suspension

A bridge to patient compliance

When a patient's inability—or unwillingness—to swallow a tablet compromises the treatment of an anaerobic or trichomonal infection, you can now turn to Flagyl-S. It is an easy-to-swallow suspension that extends the availability of effective treatment to all patients, including the elderly, the debilitated and the very young.

---

single 50ml dose in trichomoniasis

---

rescribing Information: Presentation: Suspension: each 5ml containing 320mg benzoylmetronidazole, equivalent to 200mg metronidazole. **Indications**: For the treatment of urogenital trichomoniasis in the female nd in the male. Treatment of infections in which anaerobic bacteria have been identified or are suspected as pathogens. *Adult dosage*: 50ml Suspension as a single dose. Alternatively, 5ml three times daily for seven ays, or 20ml in the morning and 30ml in the evening for two days. **Contra-indications:** There are no absolute contra-indications to the use of Flagyl. **Precautions**: Avoid alcohol or use in pregnancy, lactation may hance the effect of oral anti-coagulants. The prescriber should also bear in mind the possibility that an accompanying gonococcal infection might persist in a symptomless state after trichomonas vaginalis has been ted. **Side-effects**: Occasional unpleasant taste, furred tongue and gastro-intestinal disturbances. Drowsiness, dizziness, headache, ataxia, skin rashes, pruritus, inco-ordination of movement, darkening of urine metabolite) has been reported but very rarely. A few instances of peripheral neuropathy have been reported during intensive and/or long-term treatment. **Presentation/Cost**: Suspension Bottle of 2.05. Bottle of 125ml @ £4.80. (April 1981) PL0012/0131. Further information available on request.

Genito-urinary Medicine Technician

c.£13,000 p.a. tax free

In Jeddah, on Saudi Arabia's west coast, a 500-bed hospital is being commissioned. Built to the highest international standards, it incorporates some of the most advanced medical equipment available in the world today for all aspects of diagnosis and treatment.

It is now being managed by IHG International Hospitals Group, the British-based health care organisation which has given IAL the task of helping to staff the complex to its full complement of 2,500 people.

This is an exceptional health care project. It therefore demands an exceptional level of expertise from the nursing and paramedical staff who join the team now to put this hospital into action.

The basic qualification is an SRN – possibly SEN – certificate, followed by six months approved GU training and some post-certification experience.

A tax-free salary will be paid in Saudi Riyals*. Benefits include 49 days holiday per year, free return flights to the UK, medical care and free accommodation furnished to the highest standards. Medical City, a purpose-built, landscaped living complex adjacent to the hospital, includes shops, gymnasium, theatre, swimming pool, tennis courts and restaurants for the exclusive use of staff and their families.

*The conversion to sterling has been effected at the rate of SR6.5 = £1.

For further details please write with C.V. to: Elaine Wyatt, IAL,
Aeradio House, Hayes Road, Southall, Middlesex UB2 5NJ.
Telephone: 01-574 4960. Please quote Ref. M190.

IAL

MEDICAL SERVICES
COMMUNICATIONS SYSTEMS
COMPUTER SYSTEMS AND SERVICES
AVIATION SYSTEMS AND SERVICES-WORLDWIDE

Br J Vener Dis 1982; **58**: 1-11

Morphological destruction of cultured cells by the attachment of Treponema pallidum

T J FITZGERALD,* L A REPESH,† AND S G OAKES‡

*From the Departments of *Medical Microbiology and Immunology, †Biomedical Anatomy, and ‡Physiology, School of Medicine, University of Minnesota, Duluth, Minnesota, USA*

SUMMARY The incubation of *Treponema pallidum* with rabbit testicular cells, HEP-2 cells, human foreskin cells, rat cardiac cells, and rat skeletal muscle cells caused morphological disruption of these cultured cells. Control preparations of heat-inactivated treponemes, a high-speed supernatant in which treponemes had been pelleted, and culture medium failed to damage the tissue cells, as did viable treponemes when the cells were incubated in inverted Sykes-Moore chambers. Thus, cellular disruption is not associated with soluble treponemal, soluble inflammatory, or soluble testicular constituents but is mediated by the specific attachment of *T pallidum*. This organism apparently elaborates some type of toxic activity that lyses membranes; this may explain some of the histopathology of syphilitic disease.

Introduction

Treponema pallidum apparently lacks the potent toxins that many other bacteria possess.[1 2] Early in infection relatively large numbers of treponemes are present within infected tissue and yet cause minimal tissue damage.[1] It has been suggested that *T pallidum* is rather innocuous and that clinical manifestations are primarily due to host defences such as inflammation and clearance of organisms.[3] No specific treponemal toxins have yet been identified.

Wright[4] reported that *T pallidum* attached to tissue culture cells. Fitzgerald *et al*[5] confirmed this preliminary finding using cells from rabbit testes, which significantly extended the in-vitro survival of the treponemes. In further studies[6] *T pallidum* was shown to attach to 19 different cultured cell types and attachment was identical to that in specimens taken from rabbit dermal and testicular infections. Since non-pathogenic treponemes failed to attach to cultured cells, Fitzgerald *et al*[6 7] suggested that attachment indicated a specific pathogenic factor that was an important part of the disease process. Numerous other reports have described the *T pallidum*-tissue culture interaction.[8] Over 40 different types of cultured cells have been tested, and in each case treponemal attachment did not harm the cultured cells. As many as 50 organisms may be attached per cell and active motility of the organisms retained for 5-7 days. The cells are not morphologically altered and viability remains high.[6 9]

Repesh *et al*[10 11] using high concentrations of *T pallidum* demonstrated destruction of cultured nerve cells from dorsal root ganglia. Cell damage depended on the attachment of viable treponemes. Heat-inactivated organisms failed to attach and affect the morphology of the neuronal cells. Similarly, the cells were not damaged by a preparation containing soluble treponemal, soluble inflammatory, and soluble testicular constituents. This paper expands these observations of treponemal toxic activities to other types of cultured cells.

Materials and methods

TREPONEMA PALLIDUM

The Nichols strain was passaged intratesticularly in Dutch belt rabbits. Animals were maintained at 18°C and given antibiotic-free food and water in unrestricted quantities. Each testis was inoculated wth 1×10^7 to 3×10^7 treponemes. Daily intramuscular injections of cortisone acetate (6 mg/kg) were given. After a satisfactory orchitis had developed in 9-11 days, the rabbits were killed and the testes removed, minced with scissors, and extracted on a rotary shaker at room temperature in tissue culture medium. This medium (MEM) contained Eagle's minimal essential medium supplemented with 4 mmol/l $NaHCO_3$, 30 mmol/l HEPES

Address for reprints: Dr T J Fitzgerald, Department of Medical Microbiology and Immunology, School of Medicine, University of Minnesota, Duluth, Minnesota 55812, USA

Accepted for publication 14 September 1981

(N-2-hydroxy-ethylpiperazine-N-2-ethanesulfonic acid), 1 mmol/l dithiothreitol, and 10% heated normal rabbit serum (at 56°C for 30 minutes). After extraction for 20-25 minutes, the preparation was centrifuged at 1000 × g for 10 minutes to sediment particulate matter. Treponemes were then centrifuged at 17 000 × g for 30 minutes at 4°C. The high-speed supernatant was removed and the pelleted organisms were resuspended at a concentration of 9×10^7 to 2×10^8 organisms/ml in fresh medium.

TISSUE CULTURE CELLS

Normal rabbit testicular cells were cultured and maintained as described.[6] HEP-2 cells and cells from human foreskin (kindly provided by Dr Richard J Ziegler, University of Minnesota) were grown on glass coverslips in Sykes-Moore chambers.[12] Except for dithiothreitol, the culture medium was identical to that used for treponemal extraction. Experiments using these three cell types were performed at 10-20% confluency.

Ventricular heart tissues were obtained from 17-day-old rat embryos by the methods of Marvin *et al.*[13] Ventricles were placed into M199 medium containing Earle's balanced salts, 15% horse serum, penicillin, streptomycin, and gentamicin. The tissue was minced with scissors and exposed to four 30-minute incubations in trypsin at 37°C. The cells were placed in horse serum at 4°C, centrifuged at 200 × g for eight minutes, washed, resuspended in M199 medium with supplements of glutamine, minimal essential medium vitamin mixture, and dextrose, and incubated in 35-mm plastic culture dishes at 37°C in an atmosphere of 5% carbon dioxide. Culture medium was changed on days 2 and 5 and then weekly.

Skeletal muscle tissues, obtained from pectoral muscles of 11-day-old rat embryos by the methods of Fischbach,[14] were cut with scissors, added to trypsin, and incubated at 37°C for 30 minutes. Cells were centrifuged at 200 × g for eight minutes and resuspended in culture medium containing Eagle's minimal essential medium supplemented with 600 mg % glucose, 2% chick embryo extract, 10% heated horse serum, 10% fetal bovine serum, and gentamicin. Approximately 10^6 cells in 3 ml were placed in 60-mm plastic culture dishes. After incubation for 30-45 minutes at 37°C, cells in suspension were removed, plated in collagen-coated 35-mm dishes, and incubated at 37°C in 5% carbon dioxide. Many of the fibroblasts were eliminated by exposure to 10^{-5} mol/l D-arabinofuranosylcytosine for 48 hours on days 2 and 3. This medium was replaced with fresh culture medium without fetal bovine serum and then changed twice weekly. Experiments with heart and skeletal tissues were performed at 80-100% confluency.

The cardiac and skeletal muscles were grown in the presence of antibiotics. Before the treponemes were added, cells were washed three times with 4 ml of MEM. The five types of cultured cells were incubated at 37°C in an atmosphere of 2·5% O_2, 5% CO_2, and 92·5% N_2 with the following: viable treponemes and heat-inactivated treponemes (56°C for 10 minutes), both at concentrations of 9×10^7 to 2×10^8 organisms/ml, the high-speed supernatant obtained by pelleting the treponemes at 17 000 × g for 30 minutes, and the MEM. Each experiment was repeated 3-5 times with treponemes obtained from different animals.

SCANNING ELECTRON MICROSCOPY

The cultured cells grown as above on glass coverslips or in plastic Petri dishes were rinsed twice with Hank's balanced salt solution and fixed in situ with 3% glutaraldehyde in 0·1 mol/l sodium cacodylate buffer (pH 7·35) at room temperature for one hour. After being rinsed three times with 5 ml of 0·1 mol/l sodium cacodylate buffer containing 7·5% sucrose, the cultures were post-fixed in 1% osmium tetroxide with 0·1 mol/l sodium cacodylate for 30 minutes, washed three times in double distilled water, stained in 2% uranyl acetate and dehydrated in aqueous grades of alcohol to 100%. The samples on portions of the glass coverslips or the plastic dishes were critical-point dried using CO_2, mounted on aluminium stubs with double-stick tape, and coated with gold-palladium using a Hummer II evaporator. Observations were made with an AMR 1000 scanning electron microscope (20 kV).

Results

NORMAL RABBIT TESTICULAR CELLS

Normal rabbit testicular cells were incubated in Sykes-Moore chambers with viable treponemes, heat-inactivated treponemes, the high-speed supernatant from the infected testes, or MEM. As reported,[5] viable treponemes immediately attached to the cultured cells. Within a few minutes 10-20 organisms, and within a few hours over 100 organisms, were attached to each cell. As expected, heat-inactivated treponemes failed to attach.[5] After 24 hours, all cells exposed to viable organisms showed morphological changes, which became more pronounced after 48 hours (fig 1a). These included excessive vacuolation, loss of nuclear detail, rounded appearance, and detachment from the glass substratum. The treponemes became detached from the damaged cells. In contrast, cultured cells exposed to the three control preparations appeared normal (fig 1b).

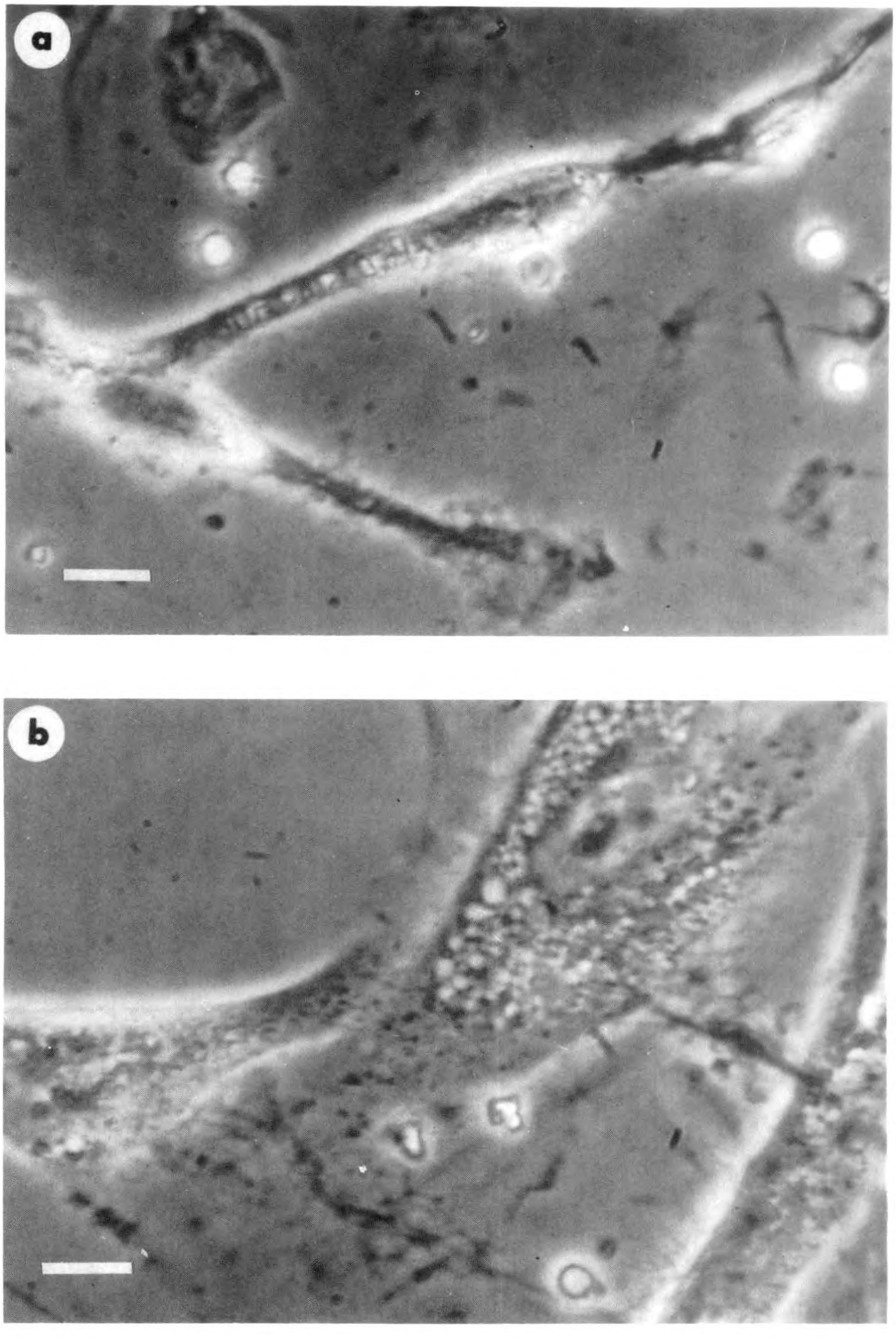


FIG 1 *Phase-contrast micrographs of cells derived from normal rabbit testicular tissue after incubation for 48 h with: (a) viable treponemes and (b) heated treponemes. Scale bar = 30 μm.*

In these experiments one additional chamber was inoculated with viable treponemes and incubated inverted so that the cultured cells were on the top. After 48 hours most of the treponemes had settled to the bottom of the chamber and relatively few organisms were attached to the cultured cells. After 48 hours the morphology of the testicular cells was unchanged and appeared identical to the cells incubated with heated treponemes, the high-speed supernatant, or the MEM. Retention of treponemal motility in chambers incubated in the inverted and upright positions was identical. Thus, the altered cellular morphology was attributed to attachment of organisms rather than to the release of some soluble treponemal factor during incubation.

HUMAN FORESKIN AND HEP-2 CELLS

In identical experiments with human foreskin cells and HEP-2 cells incubated with viable treponemes, large numbers of organisms attached to both types of cells; after 48 hours morphological damage was indicated by loss of cellular detail, rounding of cells, and cellular detachment from the substratum (fig 2a). In contrast, however, both types of cells exposed to the three control preparations retained normal morphology (fig 2b). Neither foreskin nor HEP-2 cells were morphologically damaged when inoculated with viable organisms and incubated in inverted chambers.

CARDIAC MUSCLE CELLS

These cultured cells retain the ability to beat in vitro for 2-3 weeks and numerous groups of 5-10 cells beat synchronously. Viable *T pallidum* disrupted the cell-beating process; the cardiac cells beat spasmodically with loss of synchrony and then stopped beating altogether. In preliminary experiments, beating stopped within 14 hours in the presence of 4×10^8 treponemes/ml and within 39 hours in the presence of 4×10^7 treponemes/ml. With lower concentrations of treponemes or with MEM alone, beating continued for 70 hours, when the experiments were terminated.

Morphological damage was assessed by incubating the cardiac cells with viable treponemes (9×10^7 to 2×10^8 organisms/ml), heated treponemes, the high-speed supernatant, or MEM. As with other cell types viable, but not heated, treponemes immediately attached to the cardiac cells. After 48 hours the viable preparation severely damaged the cultured cells (fig 3a). The three control preparations, however, showed normal morphology. Fig 3b shows cardiac cells incubated for 48 hours in the presence of heated treponemes. In these experiments beating of the cells stopped in the presence of the viable organisms well before morphological damage was evident.

SKELETAL MUSCLE CELLS

These muscle cells are readily differentiated from the background fibroblasts. Individual myoblasts coalesce to form characteristic long myotubes containing multiple nuclei. These cells were incubated with viable treponemes, heated treponemes, the high-speed supernatant, or MEM. The viable, but not the heated, organisms attached to the muscle cells and to the background fibroblasts. Attachment resulted in morphological destruction.

By scanning electron microscopy cultured cells exposed to the three control preparations showed normal morphology after 48 hours' incubation. In cells exposed to the high-speed supernatant for 48 hours the myotubes were elongated and characteristically grew on top of the background fibroblasts (fig 4). The myotube surface was smooth with gently rounded contours; the background fibroblasts were apparent beneath this portion of the myotube. In muscle cells exposed to viable treponemes for 24 hours the myotubes showed various stages of degradation resulting in pleomorphism; some were beginning to retract and others had a rounded morphology (fig 5a and b). After 48 hours the muscle cell (at the right of the micrograph) is apparently retracting and that on the left is almost completely rounded and contains numerous surface projections not present on cells in control preparations. At this magnification treponemes can be seen. Initially, large numbers of actively motile organisms attached along the full length of the myotubes. Morphological damage to the muscle cells resulted in detachment of treponemes.

The treponemes also damaged the background fibroblasts, causing irregular holes (fig 6); numerous attached treponemes are also evident. The underlying substratum can be seen through these damaged areas of the fibroblasts. In the three control preparations, the morphology of the background fibroblasts was unaltered.

One further notable observation was the presence of a few treponemes attached to the cultured muscle cells which were exposed to the high-speed supernatant. These treponemes were either apparently not pelleted during the high-speed centrifugation or inadvertently resuspended during the removal of the supernatant. In fig 7 a portion of a myotube is shown with an attached "half treponeme" (arrow), which appears to be penetrating into the myotube. This organism is approximately 5μm in length and has 2-3 spirals; the other (arrowhead) is approximately 20 μm in length and has 12-14 spirals.

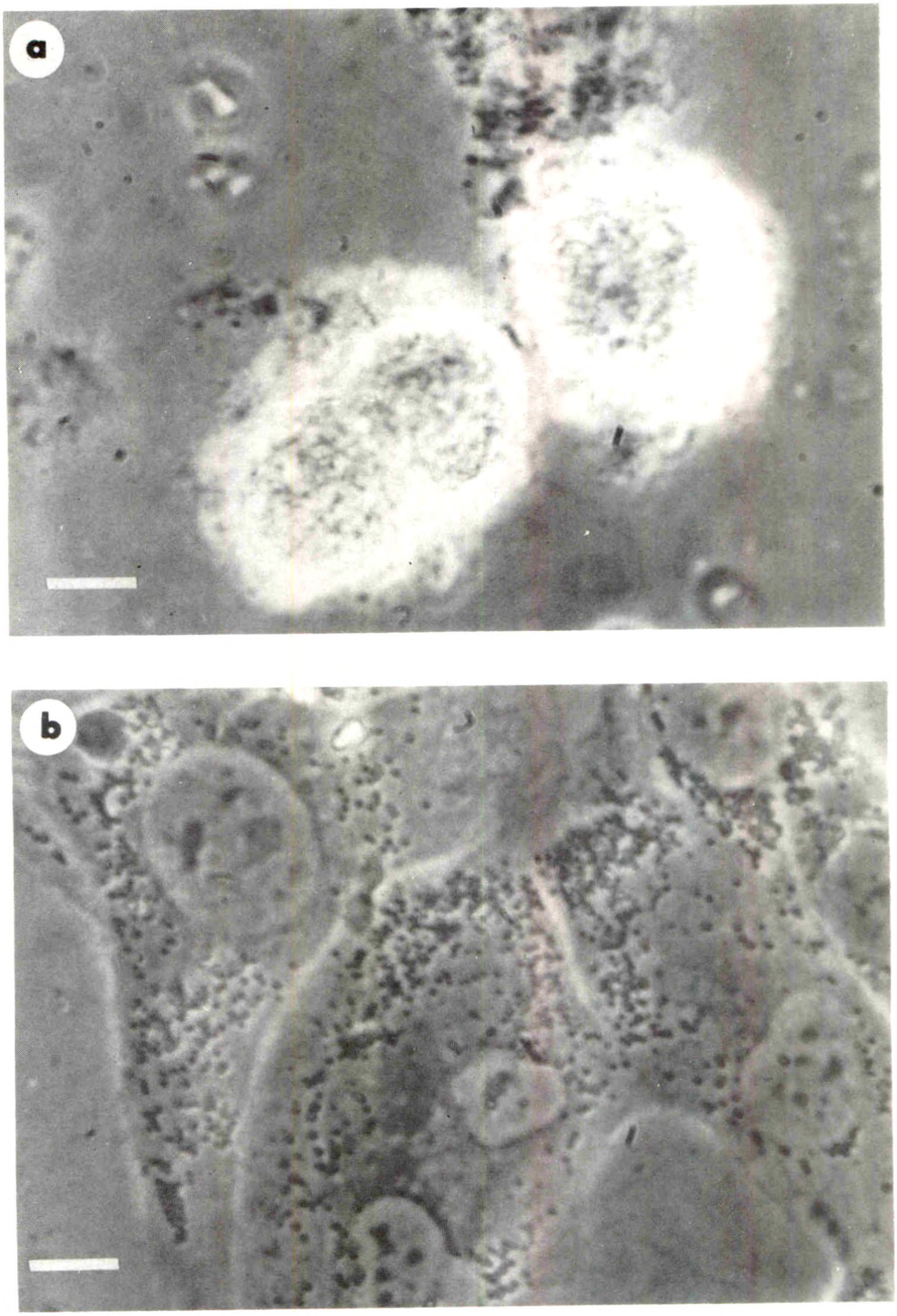


FIG 2 *Phase-contrast micrographs of HEP-2 cells after incubation for 48 h with: (a) viable treponemes and (b) MEM. Scale bar = 30 μm.*

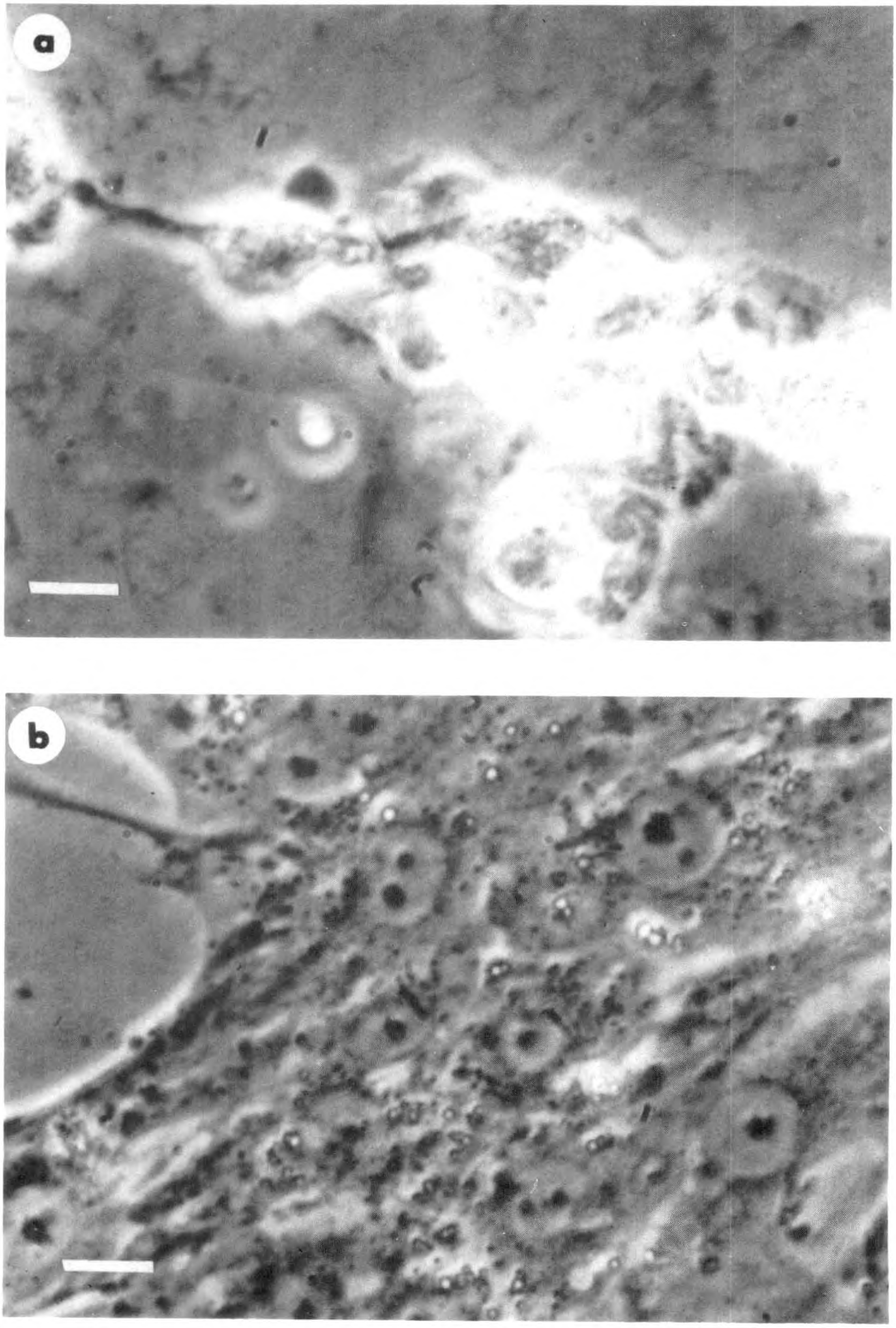


FIG 3 *Phase-contrast micrographs of rat cardiac muscle cells after incubation for 48 h with: (a) viable treponemes and (b) heated treponemes. Scale bar = 30 μm.*

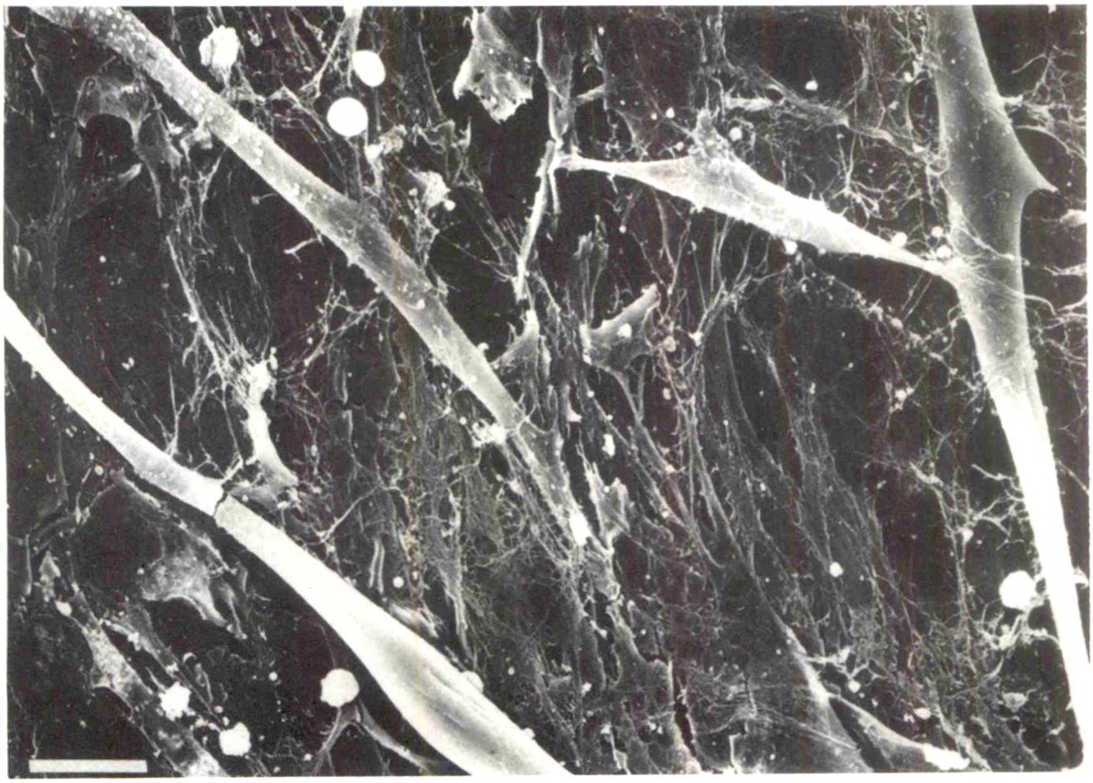


FIG 4 *Scanning electron micrograph of rat skeletal muscle cells after incubation for 48 h with the high-speed supernatant. Individual myoblasts coalesced to form elongated myotubes. Scale bar = 30 μm.*

Discussion

Repesh *et al*[10 11] reported that *T pallidum* disrupted the integrity of cultured cells from dorsal-root ganglia. The present findings extend treponeme-mediated tissue culture damage to five other cell types. Many other studies of *T pallidum*-cultured cell interaction have been performed.[8] Cellular damage had not been previously reported, probably because lower initial inocula ranging from 1 × 10^5 to 5 × 10^7 organisms/ml were used. In this study the inocula ranged from 9 × 10^7 to 2 × 10^8 organisms/ml. These concentrations are within physiological levels. After dermal inoculation of rabbits, clinical manifestations first become apparent when treponemes reach a critical concentration of 1 × 10^7 organisms.[2] With a generation time of 30 hours approximately 2·4 × 10^8 organisms should be present after five days. Since virtually every cultured cell had attached treponemes, damage was uniformly evident. Some cells, however, were more quickly damaged than others; furthermore, damage resulted in detachment of treponemes.

These studies were performed with cultured cells from rabbit, human, and rat tissues. *T pallidum* infection of rabbits mimics infection of man. In contrast, infection of rats produces minimal tissue response and asymptomatic infection.[2] Although overt clinical lesions usually do not occur, and treponemes are difficult to visualise in the tissues, their presence can be demonstrated by transfer of rat tissue to rabbits with the subsequent development of lesions. The interaction of *T pallidum* with cultured cells from rabbit and human tissue is comparable to that for other cells derived from rat, mouse, dog, monkey, and pig.[8] Similar numbers of organisms attached, and attachment extended survival. Thus, the basis for minimal treponemal infection of these animals is probably not associated with innate differences in tissues. More likely, the lack of symptomatic infection indicates innate differences in serum factors or in enhanced host humoral or cellular immune responses. Thus, insights into the pathogenesis of *T pallidum* should result from studies of cultured cells derived from different species of animals.

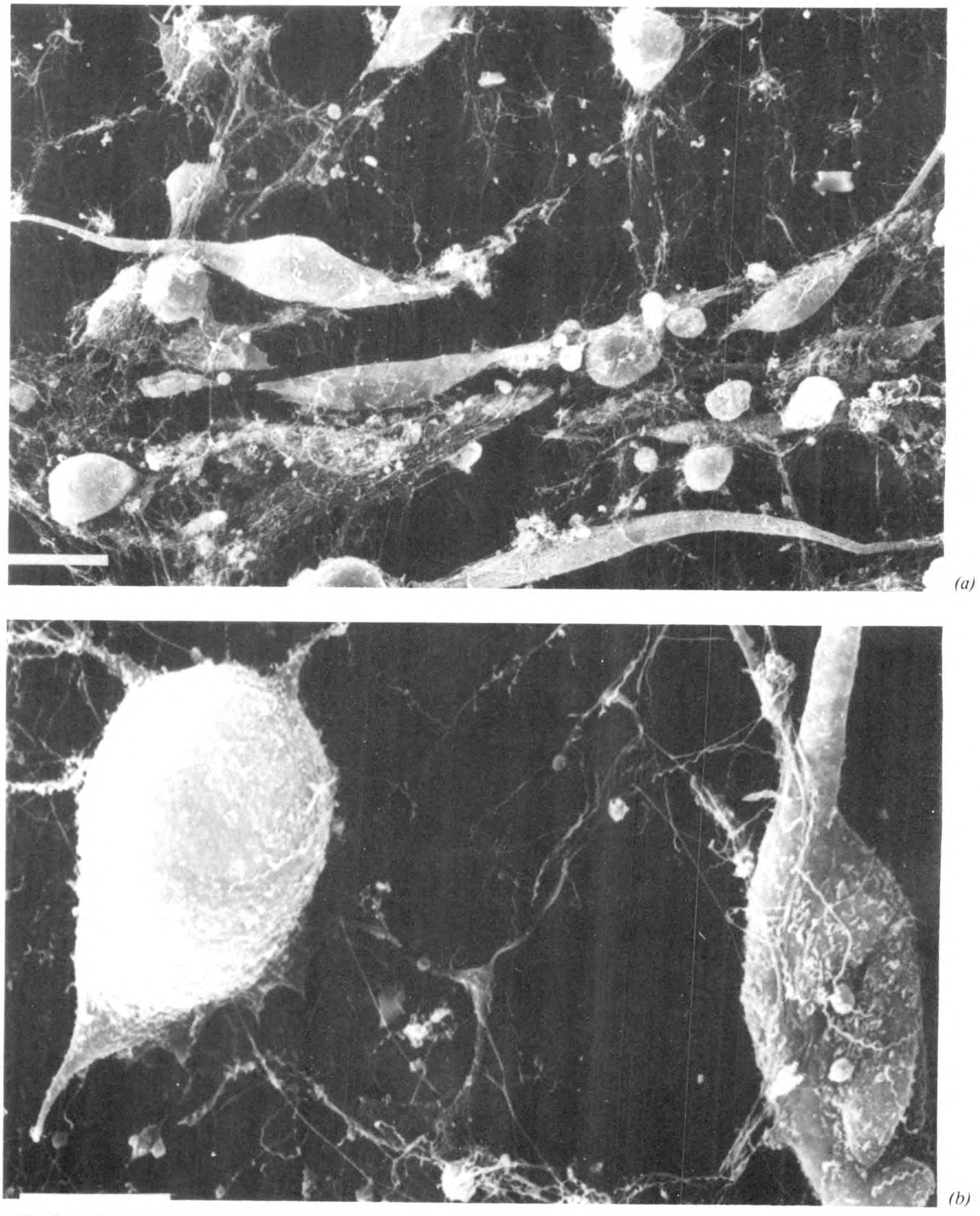


FIG 5 *Scanning electron micrographs of rat skeletal muscle cells after incubation for 24 h with viable treponemes. (a) The myotubes exhibit various stages of degradation resulting in pleomorphism. (b) After 48 h more damage is apparent; the left cell is almost completely rounded and contains surface projections not observed in control preparations. Scale bar = 30 μm (a) and 10 μm (b).*

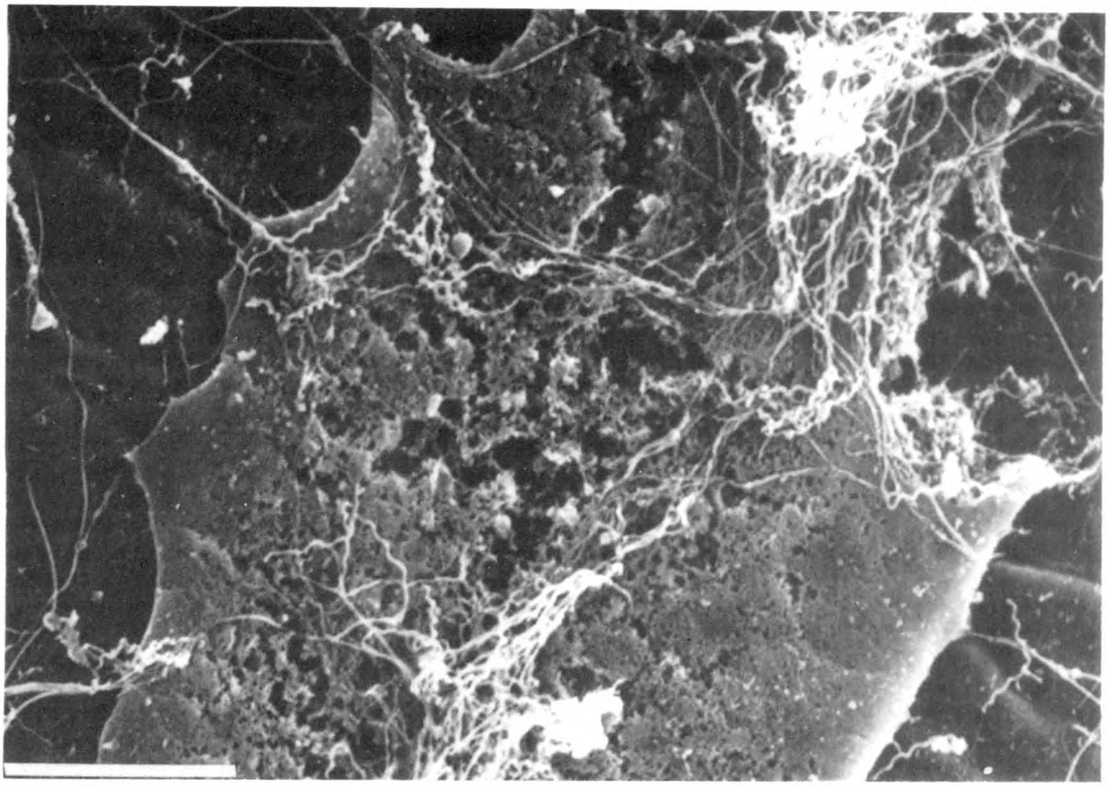


FIG 6 *Scanning electron micrograph of the background fibroblasts within the cultured skeletal muscle cell preparation after incubation for 48 h with viable treponemes. Irregular holes are apparent at the surface of this group of fibroblasts. Scale bar = 10 μm.*

Disruption of cultured cells was mediated through the attachment of viable treponemes, which had been pelleted by centrifugation and then resuspended in fresh MEM. Heat-inactivated organisms that failed to attach did not damage the cells. Soluble factors were not involved. The high-speed supernatant that remains after pelleting the organisms contains soluble treponemal, soluble inflammatory, and soluble testicular components; it did not affect the tissue culture cells. Furthermore, when chambers were inoculated with viable *T pallidum* and incubated inverted with the cultured cells uppermost, no cell damage was observed indicating that soluble factors released by the organisms during incubation were not responsible for the damage. Thus, the initial cellular lesion probably occurs at the point of attachment of treponemes. Some type of treponemal toxic activity may exert a membrane lytic effect.

T pallidum possesses the ability to penetrate cells, as shown by the partial entry of the organism into the muscle cells. It is well established that treponemes are found intracellularly in relatively low numbers in vivo within a variety of host cells[15-18] and in vitro in cultured rabbit testicular cells.[5] Penetration of treponemes may be related to their membrane lytic action resulting in small holes in the membrane. If few organisms attach, cells may be able to repair the damage. If large numbers of organisms attach, the holes may accumulate to the point of pronounced membrane damage, thereby explaining morphological disruption of the cultured cells.

Treponemes cause dysfunction of the beating of cardiac muscle cells; this occurred before morphological damage and may have reflected the early events associated with cellular disruption. A similar observation was reported with the cultured nerve cells,[10 11] in which *T pallidum* initially caused dysfunction as indicated by their loss of ability to respond to electrophysiological stimuli; this was followed by cellular disruption.

Other reports have described damage to tissue culture cells by bacteria. Portnoy *et al*[19] found that *Yersinia enterocolitica* initially attached to HEP-2 cells, then became intracellular. Within 90 minutes of

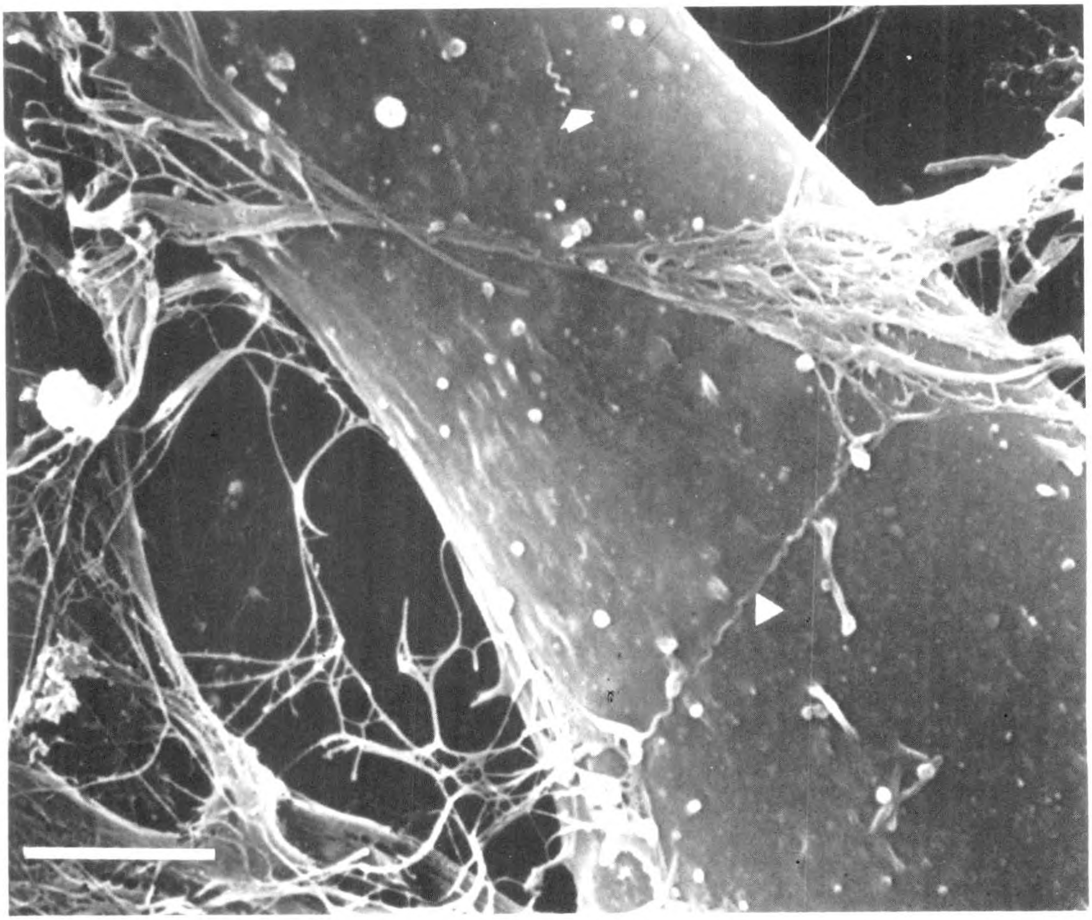


FIG 7 *Scanning electron micrograph of a portion of a skeletal muscle myotube showing a half-treponeme (arrow). Note the normal size treponeme (arrowhead). Scale bar = 5 μm.*

inoculation the cultured cells detached from the growth substratum. This toxic activity required the attachment of the bacteria to the HEP-2 cells. Neither a cell-free supernatant nor a membrane-filtered sonicate of the bacteria was detrimental. Guerrant *et al*[20] reported that the enterotoxins of both *Escherichia coli* and *Vibrio cholerae* morphologically altered Chinese hamster ovary cells within 24 hours. They suggested that cyclic adenosine monophosphate (AMP) mediated the changes, which included cell elongation, polarisation, and loss of knob-like projections. Vicari *et al*[21] demonstrated the detrimental effects of *Shigella dysenteriae* after attachment to cultured cells. Others[22-24] have confirmed and extended these findings. The *Shigella* cytotoxin specifically inhibited protein synthesis within HeLa cells and human embryonic intestinal cells. This resulted in rapid detachment of the cultured cells from the growth substratum.

Treponemal damage to tissue culture cells may explain some of the clinical manifestations of syphilis; at least two mechanisms are concerned in potential tissue pathology. Firstly, the organisms may be relatively innocuous, and damage could result from host inflammatory reactions and from host defence mechanisms involved in clearance of the organisms.[3] This mechanism is supported by the rebound phenomenon in rabbits infected dermally and treated daily with cortisone.[1,2] Large lesions develop atypically with minimal cellular infiltration and become greatly intensified a few days after withdrawal of the cortisone. The latter stimulates the host defences and produces cellular infiltration. The subsequent degradation and clearance of organisms may then explain the intensification of the lesions. Secondly, specific treponemal components may degrade various tissues. In the above example, rabbit host defences are minimised before cortisone withdrawal, and large lesions develop in the absence of the usual cellular infiltration. If the in-vitro findings presented in this paper can be extended to infection in vivo toxic activities of *T pallidum* could explain the histopathology of syphilis. In summary, both mechanisms of tissue pathology may be operative in syphilis.

We wish to acknowledge the efforts of Eileen Gannon, Bonnie Tyson, and Paul Lima in providing excellent technical assistance, Carolyn Gabriel for developing and processing the photomicrographs, and Dr Robert Pozos, Department of Physiology, University of Minnesota, Duluth, for providing the cultured cardiac cells and the cultured skeletal muscle cells. This research was supported by Public Health Service National Institute of Allergy and Infectious Diseases grant AI 16585, Minnesota Medical Foundation grant DMRF 53-80, and the University of Minnesota Graduate School grant 464-0325-4909.

References

1. Turner TB. Syphilis and the treponematoses. In: Mudd S (ed). *Infectious Agents and Host Reactions.* Philadelphia: W B Sanders, 1970:346-90.
2. Turner TB, Hollander DH. Biology of the treponematoses. *WHO Monograph Series No 35,* 1957.
3. Hardy PJ, Graham DH, Nell EE, Dannenberg AM. Macrophages in immunity to syphilis: suppressive effect of concurrent infection with *Mycobacterium bovis* BCG on the development of syphilitic lesions and growth of *Treponema pallidum* in tuberculin-positive rabbits. *Infect Immun* 1979;**26**:751-63.
4. Wright MI. Exploratory studies in tissue culture of *T pallidum. Proceedings of the XII International Congress of Dermatology* 1962;**2**:844-87.
5. Fitzgerald TJ, Miller JN, Sykes JA. *Treponema pallidum* (Nichols strain) in tissue cultures: cellular attachment, entry, and survival. *Infect Immun* 1975;**11**:1133-40.
6. Fitzgerald TJ, Johnson RC, Miller JN, Sykes JA. Characterization of the attachment of *Treponema pallidum* (Nichols strain) to cultured mammalian cells and the potential relationship of attachment to pathogenicity *Infect Immun* 1977;**18**:467-78.
7. Fitzgerald TJ, Miller JN, Sykes JA, Johnson RC. Tissue culture and *Treponema pallidum.* In: Johnson RC (ed). *The Biology of Parasitic Spirochetes.* New York: Academic Press, 1976:57-64.
8. Fitzgerald TJ. Pathogenesis and immunology of *Treponema pallidum. Ann Rev Microbiol* 1981;**35**:29-54.
9. Fitzgerald TJ, Cleveland P, Johnson RC, Miller JN, Sykes JA. Scanning electron microscopy of *Treponema pallidum* (Nichols strain) attached to cultured mammalian cells. *J Bacteriol* 177;**130**:1333-44.
10. Oakes SG, Repesh LA, Pozos RS, Fitzgerald TJ. Electrophysiologic dysfunction and cellular disruption of sensory neurons during incubation with *Treponema pallidum. J Neuropathol Exp Neurol* 1981 (in press).
11. Repesh LA, Fitzgerald TJ, Oakes SG, Pozos RS. Scanning electron microscopy of the attachment of *Treponema pallidum* to nerve cells in vitro. *J Neuropathol Exp Neurol* 1981 (in press).
12. Sykes JA, Moore EB. A simple tissue culture chamber. *Tex Rep Biol Med* 1960;**18**:288-97.
13. Marvin WJ, Robinson RB, Hermsmeyer K. Correlation of function and morphology of neonatal rat and embryonic chick cultured cardiac and vascular muscle cells. *Circ Res* 1979; **45**:528-39.
14. Fischbach GD. Synapse formation between dissociated nerve and muscle cells in low-density cell cultures. *Dev Biol* 1972; **28**:407-29.
15. Azar HA, Pham TD, Kurban AK. An electron microscopic study of a syphilitic chancre. *Arch Pathol* 1970;**90**:143-50.
16. Lauderdale V, Goldman JN. Serial ultrathin sectioning demonstrating the intracellularity of *T pallidum. Br J Vener Dis* 1972;**48**:87-99.
17. Sykes JA, Miller JN. Intracellular location of *Treponema pallidum* (Nichols strain) in the rabbit testis. *Infect Immun* 1971;**4**:307-14.
18. Sykes JA, Miller JN, Kalan AJ. *Treponema pallidum* within cells of a primary chancre from a human female. *Br J Vener Dis* 1974;**50**:40-4.
19. Portnoy DA, Moseley SL, Falkow S. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. *Infect Immun* 1981;**31**:775-82.
20. Guerrant RL, Brunton LL, Schnaitman TC, Rebhun LI, Gelman AG. Cyclic adenosine monophosphate and alteration of Chinese hamster ovary cell morphology: a rapid sensitive in-vitro assay for the enterotoxins of *Vibrio cholerae* and *Escherichia coli. Infect Immun* 1974;**10**:320-7.
21. Vicari G, Olitzki AL, Olitzki A. The action of thermolabile toxin of *Shigella dysenteriae* on cells cultivated in vitro. *Br J Exp Pathol* 1960;**41**:179-89.
22. Brown JE, Rottman SW, Doctor BP. Inhibition of protein synthesis in intact HeLa cells by *Shigella dysenteriae* 1 toxin. *Infect Immun* 1980;**29**:98-107.
23. Keusch FT. Pathogenesis of shigella diarrhea. III Effects of shigella enterotoxin in cell culture. *Trans N Y Acad Sci* 1973; 35:51-8.
24. Olsnes S, Eikled K. Isolation and characterization of *Shigella shigae* cytotoxin. *J Biol Chem* 1980;**225**:284-9.

Br J Vener Dis 1982;**58**:12-7

Demonstration of specific 19S(IgM) antibodies in untreated and treated syphilis

Comparative studies of the 19S(IgM)-FTA test, the 19S(IgM)-TPHA test, and the solid phase haemadsorption assay

F MÜLLER* AND E-G LINDENSCHMIDT†

*From the *Division of Serology, Department of Medical Microbiology, Institute of Hygiene, Hamburg; and the †Institute of Medical Microbiology and Immunology, University of Hamburg, Hamburg, Federal Republic of Germany*

SUMMARY Sera from 408 patients with untreated or treated syphilis were examined by three different tests to demonstrate treponema-specific 19S(IgM) antibodies. Antibody titres in the 19S(IgM)-fluorescent treponemal antibody (FTA) test and the solid phase haemadsorption assay (SPHA) did not correlate. The 19S(IgM)-*Treponema pallidum* haemagglutination assay (TPHA) and the SPHA partly correlated based on the concentration of treponema-specific 19S(IgM) antibodies in the patient's serum. Under experimental conditions antibody titres in the 19S(IgM)-FTA test and the 19S(IgM)-TPHA correlated consistently.

For specificity and sensitivity, the 19S(IgM)-FTA test correlated best with the clinical findings in both untreated and successfully treated patients. Although the 19S(IgM)-TPHA has about the same degree of specificity, the reading of the results is technically more complicated. The specificity of the SPHA was very high. In patients with untreated syphilis, however, the SPHA is adversely affected by a high rate of false non-reactive results, since it consists of two reactions with appreciable differences in sensitivity. Thus, higher sensitivity cannot be expected in the SPHA.

An immunoadsorption technique using an adequate antigen and a specific, enzyme-labelled antiserum might provide an alternative test which is simple to perform, highly specific, and consistently sensitive.

Introduction

In the last 10 years the demonstration of specific 19S(IgM) antibodies has acquired clinical and diagnostic importance in diseases induced by both viruses and bacteria. The value of 19S(IgM) diagnostic tests in patients with syphilis has been pointed out by several workers.[1-6] In the micromethod of the 19S(IgM)-fluorescent treponemal antibody (FTA) test, described by Müller and Loa[4] and later modified by Müller and Oelerich,[7] treponema-specific 19S(IgM) antibodies in the patient's serum are separated from those of the 7S(IgG) class by gel filtration before testing. This technique avoids false-reactive as well as false non-reactive results, which can occur in the IgM-FTA-ABS test.[4 6 8 9] The 19S(IgM)-FTA test is an infallible method for detecting patients with untreated syphilis, but it requires expensive technical equipment.

Instead of using indirect immunofluorescence, the fractions of the serum after gel filtration can be investigated quantitatively by the *Treponema pallidum* haemagglutination assay (19S(IgM)-TPHA).[6] If there is any treponema-specific 19S(IgM) activity in the fractions of the first elution peak, this can be recognised in most cases.

Recently, Schmidt[10] reported results obtained in syphilitic sera by a solid-phase haemadsorption assay (SPHA). The test, which is easy to perform, is based on the solid-phase immunosorbent technique (SPIT).[11]

This paper presents the results of comparative investigations of sera from 408 patients with treated

Address for reprints: Professor F Müller, Hygienisches Institut, Gorch-Fock-Wall 15/17, D-2000 Hamburg 36, FRG

Accepted for publication 11 July 1981

and untreated syphilis at various stages of the disease using the 19S(IgM)-FTA test, the 19S(IgM)-TPHA, and the SPHA and reports experiments on the specificity and sensitivity of the SPHA technique.

Materials and methods

SYPHILITIC SERA

Sera from 82 patients with untreated primary, secondary, or tertiary syphilis, from 12 patients with reinfections with *T pallidum,* and from 314 patients with adequately treated syphilis were investigated. Nineteen of the treated patients' sera were non-reactive in the 19S(IgM)-FTA test but showed treponema-specific antibodies of the 8S(IgM) type.[12] All specimens were accompanied by a description of the current clinical symptoms or a brief clinical history.

Sera were screened by standard techniques of the TPHA in accordance with the manufacturer's directions (Fujizoki Pharmaceuticals Co Ltd, Tokyo, Japan) and by the FTA-ABS test[13]; all gave positive results. In all sera from untreated patients treponema-specific 19S(IgM) antibodies could be demonstrated by the 19S(IgM)-FTA test. The sera of adequately treated patients were non-reactive in this test. The tests were performed after storage of the sera for a maximum of 24 hours at 4°C.

FRACTIONATION OF SERA BY GEL FILTRATION

The micro-method of Ultrogel AcA 34 filtration was performed on all sera. A 0·7-ml quantity of active serum was passed through a 1·5 × 14-cm column with phosphate buffered saline (PBS), pH 7·3, 15 ms conductivity at 22°C. Fractions of 1·3 ml were collected.[7]

19S(IgM)-TPHA TECHNIQUE

After gel filtration of the serum eight fractions (starting with the peak fraction of the first elution maximum) were investigated quantitatively with Fujizoki TPHA reagents. The titre of 19S(IgM) antibodies was calculated in almost the same way as in the 19S(IgM)-FTA test. The highest titre, estimated in the region of 19S(IgM) elution, was multiplied by 6, the dilution factor produced by gel filtration.

19S(IgM)-FTA TECHNIQUE

The method for demonstrating treponema-specific 19S(IgM) antibodies in fractions after gel filtration by indirect immunofluorescence has been described.[7] The Nichols strain of *T pallidum* was used as antigen. An FITC-labelled rabbit anti-human-IgM serum with μ-chain specificity (Dako Immunochemicals, Copenhagen, Denmark) was used at a dilution of 1/70.

SOLID-PHASE HAEMADSORPTION ASSAY

The coating of the polystyrene microtitre plates, absorption time, temperature, incubation conditions, and the quality of the anti-human-IgM serum are very important preconditions for optimal SPHA results. The conditions used were comparable to those described by Schmidt.[10] Briefly, 1/400-1/800 dilutions of rabbit anti-human-IgM serum (μ-chain specific: Dako Immunochemicals, Copenhagen, Denmark) in PBS were used for coating the solid-phase in the cups of polystyrene plates. The plates were not dried before being used but were washed three times with 0·05% Tween 20 PBS. Sensitised and unsensitised sheep erythrocytes from the Fujizoki TPHA kit were used at a dilution of 1/13. Results were read after incubation for about 18 hours at room temperature. For further technical details see Schmidt.[10]

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

Preparation and peroxidase-labelling of soluble treponemal antigen

Suspensions of *T pallidum* (Nichols strain) from testes of infected rabbits, prepared in PBS and containing no cell detritus, were homogenised by ultra-sonication (Sonicator TM, Model W 185 F; 6 × 45 sec/50W at 4°C). The ultra-sonicate was centrifuged at 2500 × *g* for 20 minutes. The protein content of the supernatant (soluble *T pallidum* antigen) was estimated. According to the protein concentration the supernatant was labelled with horseradish peroxidase (HRPO, Sigma Chemical Co, St Louis, Mo, USA) by the method of Wilson and Nakane.[14]

Enzyme reaction

Forty milligrams o-phenylenediamine and 20 μl 30% hydrogen peroxide as substrate were dissolved in 100 ml citrate buffer (0·1 mol/l citric acid and 0·2 mol/l $Na_2HPO_4.2H_2O$), and 100 μl per cup were applied to perform a single enzyme reaction. After incubation for five minutes at 20°C in the dark, the reaction was stopped by the addition of 4 mol/l sulphuric acid. The colour intensity was read at 492 nm in a Multiscan (Titertek).

Results

COMPARISON OF ANTIBODY TITRES BY DIFFERENT TESTS

19S(IgM)-FTA test and SPHA

These two systems for estimating antibody titres are based on different techniques (see above). As expected the results of the 19S(IgM)-FTA test and the SPHA did not correlate. Sera with high antibody titres in the 19S(IgM)-FTA test may give SPHA results that range between non-reactive and as high as 1/512. Conversely, in our study no patient with typical symptoms of untreated primary, secondary. or tertiary syphilis whose serum was non-reactive in the 19S(IgM)-FTA test was seen.

19S(IgM)-TPHA and SPHA

Antibody titres estimated by the 19S(IgM)-TPHA and the SPHA are compared in fig 1. Of interest is the correlation of antibody titres in sera from patients with primary syphilis (and normally very low SPHA titres). Furthermore, the sensitivity of the 19S(IgM)-TPHA was three dilution steps higher than in the SPHA (fig 1).

In patients with secondary or tertiary syphilis, however, the higher the titre of treponema-specific 19S(IgM) antibodies in the serum, the closer the titres become in the two assays. In the lower range of titres the mean correlation is appreciably lowered. In the upper range of titres 19S(IgM)-TPHA and SPHA show the same sensitivity.

False non-reactive results occurred much more frequently in the SPHA, at the lower borderline values, than in the 19S(IgM)-TPHA (fig 1).

19S(IgM)-TPHA and 19S(IgM)-FTA test

The antibody titres estimated by the two assays correlated well independently of the stage of untreated syphilis (fig 2). The estimated mean values of correlation show a straight line which is parallel to the ideal correlation of titres.

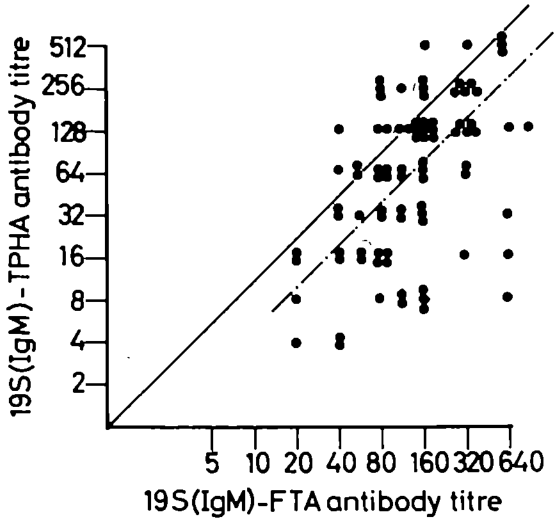


FIG 2 *Comparison of 19S(IgM)-TPHA and 19S(IgM)-FTA test antibody titres in patients with untreated primary, secondary, or tertiary syphilis. _._. Mean antibody titre.*

SPECIFICITY AND SENSITIVITY OF THE SPHA

The results of the SPHA on the sera of patients with untreated primary, secondary, and tertiary syphilis are summarised in table I. The SPHA was less sensitive when the results are compared with those of the 19S(IgM)-FTA test or the 19S(IgM)-TPHA. The rate of false non-reactive SPHA results was very high

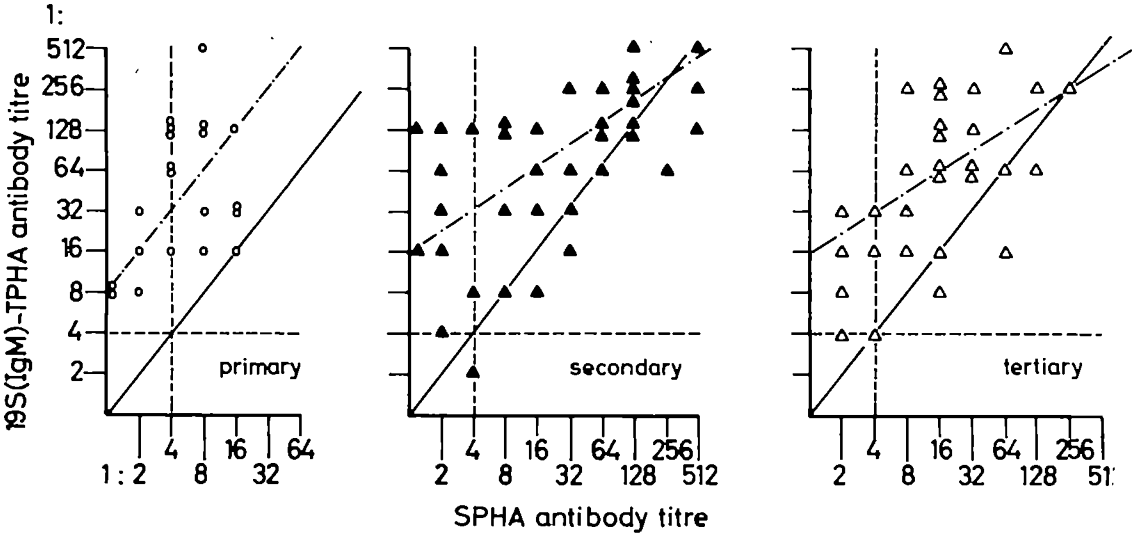


FIG 1 *Comparison of 19S(IgM)-TPHA and SPHA antibody titres in patients with untreated primary, secondary, or tertiary syphilis._ _ _ _ Borderline value of reaction specificity; _._. mean antibody titre.*

TABLE I *Solid-phase haemadsorption assay (SPHA) antibody titres in untreated patients with clinical symptoms of primary, secondary, or tertiary syphilis. (Sera that showed competitive inhibition in the IgM-FTA-ABS are not excluded.)*

19S(IgM)-FTA reactive sera from untreated patients with:	*No of sera tested*	*SPHA antibody titres*						*Non-reactive SPHA results (%)*
		<8	*8*	*16*	*32*	*>32*	*NR*	
Primary syphilis	20	9	5	4	0	0	2	11 (55)
Secondary syphilis	37	8	4	4	4	15	2	10 (27)
Tertiary syphilis	29	7	4	8	4	6	0	7 (24)
Total	86	24	13	16	8	21	4	28 (33)

*Non-reactive in the serum dilution 1/2 (before addition of sensitised sheep erythrocytes).

in the patients with primary syphilis (55%). About one-third of all the infections in patients with clinical symptoms of untreated syphilis would not have been diagnosed by the SPHA if the antibody titre of 1/8 had been used as a borderline value as described by Schmidt.[10]

All the sera from 296 patients with adequately treated syphilis gave non-reactive results in the 19S(IgM)-FTA test and in the 19S(IgM)-TPHA. None gave an SPHA titre higher than 1/4.

In the sera of 18 patients with adequately treated syphilis no 19S(IgM) but only a treponema-specific 8S(IgM) antibody could be demonstrated. As expected a positive SPHA result was found in 14 of these cases (SPHA titre ⩾1/8). In one patient the SPHA titre was 1/512 (table II).

In 12 patients reinfection with *T pallidum* was diagnosed clinically. Most of these patients' sera gave only low titres of treponema-specific 19S(IgM) antibodies by the 19S(IgM)-FTA test, but two-thirds of the sera showed false non-reactive SPHA results (table II).

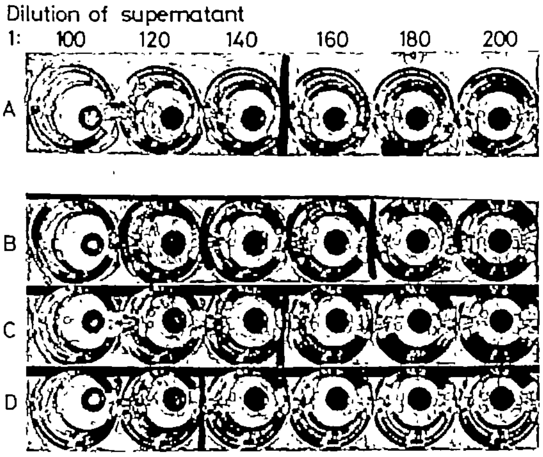


FIG 3 *TPHA of treponema-specific 19S(IgM) antibodies before (A) and after (B-D) immunoadsorption on anti-IgM-coated solid-phase.*

IMMUNOADSORPTION BY THE TPHA AND ELISA
The 19S(IgM) fraction was separated by gel filtration from the sera of three patients with untreated secondary syphilis. The TPHA titre of treponema-specific 19S(IgM) in the non-enriched eluates was estimated to be between 1/140 and 1/160 (Fig 3:A).

One hundred microlitres of an undiluted eluate, containing the treponema-specific 19S(IgM) portion of the sera, was put in the cups of polystyrene microtitre plates coated with anti-human-IgM serum (solid-phase). After three hours' incubation at 37°C in a humid chamber the supernatant fluids were

TABLE II *SPHA antibody titres in groups of patients with 8S(IgM) antibodies or treponemal reinfections*

	No tested	*SPHA antibody titres*						*False SPHA results (%)*
		<8	*8*	*16*	*32*	*>32*	*NR**	
19S(IgM)-FTA non-reactive, 8S(IgM) reactive sera from patients with adequately treated syphilis	18	4	2	3	7	2	0	14 (78)†
19S(IgM)-FTA reactive sera from patients with re-infections	12	8	2	0	0	2	0	8 (66)‡

*Non-reactive in the serum dilution 1/2 (before addition of sensitised sheep erythrocytes).
† False-positive
‡ False-negative

tested for a decrease in the level of treponema-specific 19S(IgM) antibody by the TPHA and ELISA techniques.

In the TPHA the antibody titre was not decreased. In the range of mean error the supernatants still showed a specific antibody titre of between 1/120 and 1/160 with a mean value of 1/140 (fig 3:B-D).

Despite this negative result we suspected that immunoadsorption of treponema-specific 19S(IgM) antibodies from patients' sera to the solid-phase must occur during the first reaction step. This was confirmed as follows: the supernatant fluids of the first reaction step were removed from the cups, which were then washed three times, filled with 100µl of a soluble peroxidase-labelled *T pallidum* antigen at dilutions of 1/10, 1/25, and 1/50 and incubated at 4°C overnight. After completion of the enzyme reaction there was a significant increase of extinction (at 492 nm) in the presence of antigen-conjugate dilutions of 1/10 and 1/25 compared with the results of 19S(IgM) negative cups (extinction factor $\geqslant 2$ of the mean value of negative controls in the same test).

By this experiment we demonstrated qualitatively beyond any doubt the binding of treponema-specific 19S(IgM) antibody to the solid-phase. A quantitative estimate of the extent of immunoadsorption cannot be made by this method.

Discussion

For evaluating treatment requirements in syphilis the demonstration of treponema-specific 19S(IgM) antibodies has recently aroused scientific and diagnostic interest, especially in cases of latent and late latent infections.[2-5 15-18] The IgM-FTA-ABS test, formerly used for this purpose, has been shown to be unreliable. If the patient's serum contains a rheumatoid factor, as well as treponema-specific 7S(IgG) antibodies, false-reactive results may occur. Also, false non-reactive results are possible if the fixation of the 19S(IgM) antibody to the antigen is partially or completely inhibited by an excess of 7S(IgG) antibodies of the same specificity.[8-10]

Separation of the 19S(IgM) from the 7S(IgG) fraction of serum by gel filtration before testing overcomes these limitations.[1 4-7 18] The 19S(IgM)-FTA test was found to be reliable in most patients who were untreated or who had received only inadequate treatment.[16 18] Unfortunately, this test requires highly developed techniques and can therefore be carried out only in specialised laboratories. Thus, simpler methods with similar specificity and sensitivity were looked for. Schmidt's[10] report of comparable results with the SPHA led us to continue the experiments with the SPHA which we had started some years earlier (unpublished data).

The comparative studies reported here show that the correlations of the results by the various tests do differ. No correlation could be observed between the antibody titres of the 19S(IgM)-FTA test and those of the SPHA. When the titres of the 19S(IgM)-FTA test and the 19S(IgM)-TPHA, however, and those of the 19S(IgM)-TPHA and the SPHA were compared a relationship could be found (figs 1 and 2). In the 19S(IgM)-TPHA and in the SPHA sheep erythrocytes sensitised with fragments of *T pallidum* are used as antigen. In both the 19S(IgM)-FTA test and the 19S(IgM)-TPHA the previously separated 19S(IgM) portion of the serum is tested.

The relationship between the results of the 19S(IgM)-FTA test and the 19S(IgM)-TPHA is clearly important (fig 2). The difference in titres of about one-dilution step results from the test conditions and seems to be negligible. The reading of the titre in the 19S(IgM)-TPHA is difficult, however, in some cases and impossible in others. Therefore, the 19S(IgM)-TPHA cannot be recommended for the routine detection of 19S(IgM) antibodies in syphilis.

In view of the expensive equipment needed for the highly sensitive 19S(IgM)-FTA test and the difficulties in estimating titres in the 19S (IgM)-TPHA (especially in the presence of high-titre 7S(IgG) antibodies of the same specificity), the question arises whether the sensitivity of the SPHA ensures the necessary diagnostic certainty in all syphilitic patients needing treatment. We found a false non-reactive result (titre $\leqslant 1/4$) in more than half the patients with primary syphilis and in about one-third of the patients with untreated syphilis (table I). In patients reinfected with *T pallidum* the SPHA results were unreliable in more than 75% of cases. Furthermore, the SPHA is unable to distinguish between 19S(IgM) and 8S(IgM) antibodies (table II). These observations contrast with those of Schmidt.[10] On the other hand, the high specificity of the SPHA reported by this author was confirmed in a large number of adequately treated cases.

One reason for the differences in the results may be that Schmidt[10] excluded sera that showed competitive inhibition, whereas these were included in our study. Several technical circumstances which might explain the divergence in the results have been mentioned. A fundamental factor remains to be considered, however: the SPHA consists of two immune reactions, each with an appreciable difference in sensitivity. From the experiments of Engvall and Perlmann[19] a difference in sensitivity between the immunoadsorption and indirect haemadsorption phase of about 10^3 can be expected. This fact must influence the test results.

Cantarero *et al*[20] have shown that the binding of

IgG on polystyrene in the solid-phase immunoassay does not depend on the total amount of IgG in the cups. Under normal experimental conditions about 30% of the total IgG will be fixed on the polystyrene. Our experiments agree with these observations and have shown that immunoadsorption of treponema-specific 19S(IgM) antibodies (with titres between 1/120 and 1/140) on the anti-IgM solid-phase cannot be detected by haemadsorption (fig 3) but can be with the more sensitive ELISA technique.

These findings possibly indicate that to obtain a reactive SPHA the percentage of treponema-specific 19S(IgM) antibodies of the total 19S(IgM) in a serum sample must be very high. This is confirmed by our SPHA results in patients with secondary syphilis and by the high proportion of treponema-specific 19S(IgM) antibodies in the total serum 19S(IgM). Further confirmation of this is evident in the negative results for the other groups of untreated patients; if in the case of primary or tertiary syphilis the proportion of treponema-specific 19S(IgM) in the total 19S(IgM) is still, or has become, low, then the anti-IgM of the solid-phase is blocked mostly by treponema-unspecific 19S(IgM). The non-reactive SPHA result seems to be due to the indirect haemadsorption, which is strikingly less sensitive than the first step of the assay.

The demonstration of treponema-specific 19S(IgM) antibodies may assume considerable medical importance in the future. Tests which are simple to perform, highly specific, and consistently sensitive are therefore needed. The results of the present studies show that the SPHA does not fulfil these conditions completely. These may be achieved, however, if the antibody of the patient's serum was bound to a properly antigen-coated solid-phase by immunoreaction and identified by a highly specific, enzyme-labelled antiserum, such as in the ELISA. The application of this technique is under investigation.

References

1. Atwood WG, Miller JL Fluorescent treponemal antibodies in fractionated syphilitic sera. The immunoglobulin class. *Arch Dermatol* 1969;100:763-9.
2. Julian AJ, Logan LC, Norins LC. Early syphilis: immunoglobulins reactive in immunofluorescence and other serological tests. *J Immunol* 1969;102:1250-9.
3. O'Neill P, Nicol CS. IgM-class antitreponemal antibody in treated and untreated syphilis. *Br J Vener Dis* 1972;48:460-3.
4. Müller F, Loa PL. New possibilities in the immunological diagnosis of treponemal infection (syphilis). *Infection* 1974; 2:127-31.
5. Shannon R, Booth SD. The pattern of immunological response at various stages of syphilis. *Br J Vener Dis* 1977;53:281-6.
6. Müller F, Oelerich S. Correlation between immunological parameters and the stage of clinical apparent or latent syphilis. *Dermatol Monatsschr* 1979;165:385-95.
7. Müller F, Oelerich S. A modified technique of the IgM-FTA-19S test for the demonstration of competitively inhibited antibodies in syphilis. *Das Ärztliche Laboratorium* 1978;98:386-91.
8. Cohen IR, Norins LC, Julian AJ. Competition between and effectiveness of IgG and IgM antibodies in indirect antibody and other tests. *J Immunol* 1967;98:143-9.
9. Müller F. Zur Technik des Nachweises treponemenspezifischer 19S-IgM-Antikörper bei der latenten und spätlatenten Syphilis. *Immun Infekt* 1977;5:109-13
10. Schmidt BL. Solid-phase haemadsorption: A method for rapid detection of *Treponema pallidum*-specific IgM. *Sex Transm Dis* 1980;7:53-8.
11. Krech U, Wilhelm JA. A solid-phase immunosorbent technique for the rapid detection of rubella IgM by haemagglutination inhibition. *J Gen Virol* 1979;44:281-6.
12. Müller F, Oelerich S. Identification of a low-molecular weight IgM antibody with *Treponema pallidum* specificity in sera of patients with chronic syphilis. *Klin Wochenschr* 1979; 57:667-71.
13. Müller F. Vergleichende Untersuchungen über die Wirkung von Sorbent und *Treponema*-Ultrasonikat auf die Syphilis-Spezifität des FTA-ABS-Tests. *Das Ärztliche Laboratorium* 1976;22:531-4.
14. Wilson MB, Nakane PK. Recent developments in the periodate method of conjugating horseradish peroxidase (HRPO) to antibodies. In: Knapp W, Holubar K, Wiek G (eds). *Immunofluorescence and Related Staining Techniques.* Amsterdam: North-Holland Biochemical Press, 1978: 215-24.
15. Müller F. Serodiagnostik der Syphilis aus der Sicht des Immunologen. *Hautarzt* 1977;28:167-72.
16. Herbst B-R, Goerz G, Muller F. Diagnostischer und therapeutischer Aussagewert des IgM-FTA-ABS- und des IgM-FTA-19S-Tests bei der Syphilis. *Aktuel Dermatol* 1979; 5:175-83.
17. Fritsche D. Die Beurteilung der Behandlungsbedürftigkeit latenter Lues-Infektionen. *Aktuel Dermatol* 1978;4:107-11.
18. Schmidt B. The 19S-IgM-FTA-ABS test in the serum-diagnosis of syphilis. *WHO/VDT/Res* 1979:362.
19. Engvall E,Perlmann P. Enzyme-linked immunosorbent assay. Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulins in antigen-coated tubes. *J Immunol* 1972; 109:129-35.
20. Cantarero LA, Butler JE, Osborne JW. The adsorptive characteristics of protein for polystyrene and their significance in solid-phase immunoassays. *Anal Biochem* 1980; 105:375-82.

COLLEGE OF MEDICINE
LIBRARY

Br J Vener Dis 1982;**58**:18-22

Alterations in T lymphocytes and T-lymphocyte subpopulations in patients with syphilis

JØRGEN R JENSEN AND ELLIS FROM

From the Department of Dermatology and Venereology, University of Aarhus, Marselisborg Hospital, Aarhus, Denmark

SUMMARY The distribution of T-lymphocyte subpopulations was studied in 34 patients with primary or secondary syphilis before and after treatment. An absolute and relative T lymphopenia was found in all patients. In primary syphilis the concentration of helper cells—T cells with Fc receptors for IgM (Tμ)—was low whereas in secondary syphilis the suppressor cell concentration—T cells with Fc receptors for IgG (Tγ)—was reduced. Using lymphocytes from healthy subjects this could be imitated in vitro by the addition of serum from patients with secondary syphilis. In many autoimmune diseases a low concentration of Tγ cells may be a primary factor in the production of autoantibodies. The occurrence of similar changes in patients with secondary syphilis, however, indicates that such fluctuations in the T-cell subpopulations may take place during a strong immune response.

Introduction

In syphilis the appearance of heterologous antibodies is diagnostic of the disease and indicates polyclonal activation of B lymphocytes. At the same time various cell-mediated immune functions seem to be reduced.[1-6] These findings indicate that patients with syphilis have an imbalance between B-cell activation and T cells with immunoregulatory functions.

In the present investigation of patients with syphilis, characteristic changes were observed in the subpopulations of T lymphocytes. These changes were related to the presence of antibodies and could be reproduced in vitro. The implication of these findings is discussed in relation to similar observations in patients with autoimmune diseases.

Patients and methods

STUDY POPULATION

Thirty-four patients with syphilis were studied. Their ages ranged from 16 to 44 years (mean 27·2 years). Seventeen had primary and 17 secondary syphilis. All had positive serological test results for syphilis. After syphilis had been diagnosed clinically, blood was collected for immunological investigations. The patients were then treated with procaine penicillin retard (PAM) 600 000 IU daily for either 10 days (primary syphilis) or 14 days (secondary syphilis). The immunological investigations were repeated one and two months after treatment.

Ninety-eight controls were studied, ranging in age from 16 to 60 years (mean 29 years); these were staff members and other individuals who had been screened for venereal disease and were without actual disease at the time of the study.

ISOLATION OF MONONUCLEAR CELLS

Lymphocytes from heparinised blood were isolated on Ficoll-Hypaque gradients and phagocytic cells removed by the carbonyl iron incubation method. After separation, lymphocytes were washed three times in Hank's balanced salt solution (HBSS) with 2% fetal calf serum (FCS) and adjusted to 1×10^6/ml.

ENUMERATION OF T LYMPHOCYTES

One hundred microlitres of lymphocytes (1×10^6/ml) and 100 μl of 2-aminoethylisothiouronium bromide hydrobromide-treated sheep erythrocytes (E-AET)[7] were incubated at 37°C for 15 minutes in RPMI-1640 with 20% FCS in a ratio of 1:100. The mixture was spun at 150 × *g* for five minutes and incubated at 4°C for at least one hour. After 10 μl of 0·1% acridine orange had been added the pellet was carefully resuspended, and 200 lymphocytes were counted with a fluorescence microscope. Lymphocytes with three or more erythrocytes bound to their surface were counted as rosette-forming cells.

Address for reprints: Dr J R Jensen, Department of Dermatology and Venereology, Marselisborg Hospital, 8000 Aarhus C, Denmark

Accepted for publication 14 July 1981

The subfraction of T lymphocytes with high affinity to sheep erythrocytes form rosettes with untreated sheep erythrocytes (E-active or E-a rosettes). One hundred microlitres of lymphocytes suspended in RPMI-1640 without FCS were incubated with 100 μl untreated sheep erythrocytes in a ratio of 1:10 for five minutes at 37°C. The mixture was spun for five minutes at 150 × *g*, incubated at 4°C for five minutes, and resuspended for rosette enumeration.

PURIFICATION OF T LYMPHOCYTES

Five millilitres of E-AET and 5 ml of lymphocytes were mixed and incubated as described above. After resuspension, Ficoll-Hypaque flotation was performed (600 × *g* for 15 minutes). The pellet of E-AET-rosetting T lymphocytes was resuspended in 0·83% TRIS NH_4Cl (10 minutes) to lyse the erythrocytes. After three washes with HBSS without serum, the T lymphocytes were adjusted to a concentration of 1 × 10^6/ml. Purity, as determined by E-AET rerosetting of the T-lymphocyte suspensions, was between 91% and 98%. Lymphocytes with surface immunoglobulin (B lymphocytes) were not found in the purified T-lymphocyte suspensions.

PREPARATION OF ANTI-OX-RBC ANTIBODIES

Rabbits were immunised by weekly subcutaneous injections of 1 ml of 10% ox red cells (ORBC) in Freund's incomplete adjuvant. Bleedings were performed weekly starting one week after the first immunisation.[8] Serum was inactivated at 56°C for 30 minutes and IgG was purified by DEAE-cellulose ion-exchange chromatography. IgM was obtained by gel filtration (Ultrogel AcA 22, LKB). Both the IgG and IgM suspensions gave single peaks in a crossed immunoelectrophoresis against swine anti-rabbit immunoglobulins. Purity of the IgM suspension was further indicated by the lack of rosetting capacity of monocytes with ORBC coated with IgM antibodies; all monocytes with ORBC coated with IgG antibody, however, formed rosettes.

ERYTHROCYTE-ANTIBODY (EA) SUSPENSIONS

ORBC-antibody complexes were prepared from ox erythrocytes in Alsevers solution, washed five times in HBSS, and resuspended to a concentration of 2%. Subagglutinating concentrations of IgG or IgM anti-ORBC antibody were mixed with equal volumes of 2% ORBC and kept for 90 minutes at room temperature. Three washes of the ORBC-antibody complexes were done in HBSS with 2% FCS. Finally, 10% FCS was added. These erythrocyte-antibody (EA) suspensions were kept at 4°C and used during the next two days.

ENUMERATION OF T-LYMPHOCYTE SUBPOPULATIONS

Purified T lymphocytes were incubated in RPMI-1640 with 20% FCS at 37°C in a humidified CO_2 atmosphere. After 24 hours' (day 1) and 48 hours' (day 2) incubation appropriate amounts of T lymphocytes were washed three times in HBSS with 2% FCS. The T lymphocytes (0·01 ml) were mixed with ORBC coated with subagglutinating doses of either IgG (0·01 ml) or IgM (0·01 ml), spun immediately at 150 × *g* for five minutes, and incubated at 4°C for three hours. The percentage of cells forming rosettes was counted as described above.

SERUM ASSAYS

Immunoglobulins in serum from each of three patients with secondary syphilis were removed by ammonium sulphate precipitation. Sera from three controls were treated similarly. T lymphocytes from the three controls were cultured for 24 hours and incubated for 60 minutes at 37°C with autologous serum, autologous serum without immunoglobulins, syphilitic serum, and syphilitic serum without immunoglobulins. T lymphocytes without addition of serum acted as controls. Serum was not inactivated by heat. Cell numbers and cell deaths were controlled before and after serum treatment. Three washes with HBSS with 2% FCS were done before rosette enumeration.

STATISTICAL ANALYSIS

Student's *t* test (two-tailed) was used.

Results

The total number of T lymphocytes (E-AET rosette-forming lymphocytes) was significantly reduced during both primary and secondary syphilis (table I). This was due to lymphopenia, since the percentage of T lymphocytes was within the normal range although slightly reduced (table II).

The subfraction of T lymphocytes with high affinity for sheep erythrocytes (E-a rosette-forming lymphocytes) was also reduced (table I). In patients with primary syphilis both an absolute and relative reduction of E-a T lymphocytes was found, whereas in patients with secondary syphilis only the absolute number of E-a T cells was reduced (table II).

During primary syphilis the percentage of T lymphocytes with Fc receptors for IgM (Tμ) was significantly reduced. T lymphocytes with Fc receptors for IgG (Tγ) were slightly increased ($t_{98}=1\cdot50$, $0\cdot1<P<0\cdot2$; fig 1). In patients with secondary syphilis this was almost reversed. The

TABLE I *Total number of T lymphocytes in peripheral blood from patients with syphilis and controls*

	T cells per mm³ (mean ± SEM) (n):			
	Before treatment	*One month after treatment*	*Two months after treatment*	*Controls*
E-AET				
Primary syphilis	1479±213(9)*	2052±293(6)	2045±228(7)	2089±73·11(87)
Secondary syphilis	1579±183(13)†	2204±224(10)	2651±352(9)	
E-a				
Primary syphilis	829±63(9)‡	1175±157(7)	1061±229(6)	1179±47·24(84)
Secondary syphilis	882±83(12)§	1302±130(12)	1477±245(8)	

SEM = standard error of mean; n = No of patients or controls; E-AET = 2-aminoethylisothiouronium bromide hydrobromide-treated sheep erythrocytes; E-a = untreated sheep erythrocytes
*Significantly reduced (t_{94} = 2·71, P<0·01)
†Significantly reduced (t_{98} = 2·58, P<0·02)
‡Significantly reduced (t_{91} = 4·45, P<0·005)
§Significantly reduced (t_{94} = 3·12; P<0·005)

TABLE II *Percentage of T lymphocytes in peripheral blood from patients with syphilis and controls*

	Percentage of T cells (mean ± SEM) (n):			
	Before treatment	*One month after treatment*	*Two months after treatment*	*Controls*
E-AET				
Primary syphilis	77·4±2·5(15)	76·7±1·2(10)	78·7±1·4 (7)	80·5±0·55(98)
Secondary syphilis	77·6±2·0(16)	81·4±1·2(14)	78·3±1·5(11)	
E-a				
Primary syphilis	40·4±1·8(14)*	41·7±1·7(9)†	38·3±3·3(7)‡	45·9±0·93(95)
Secondary syphilis	44·1±2·4(15)	46·7±3·8(14)	41·0±2·1(10)	

SEM = standard error of mean; n = No of patients or controls; E-AET = 2-aminoethylisothiouronium bromide hydrobromide-treated sheep erythrocytes; E-a = untreated sheep erythrocytes
*Significantly reduced (t_{107} = 2·7, P<0·01)
†Significantly reduced (t_{102} = 2·15, P<0·05)
‡Significantly reduced (t_{100} = 2·23, P<0·05)

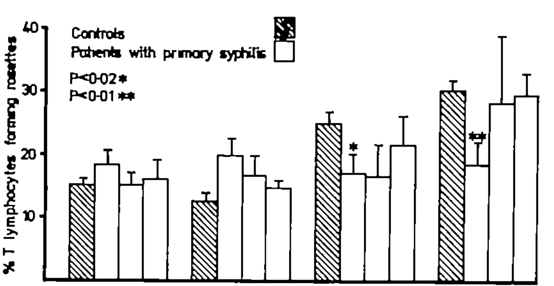


FIG 1 *The percentage of Tγ and Tμ rosettes in patients with primary syphilis before treatment (A, n = 14), one month after (B, n = 7), and two months after treatment (C, n = 7) in T cells cultured for 24 hours and 48 hours compared with controls (n = 84). Results represent mean ± SEM (Student's t test).*

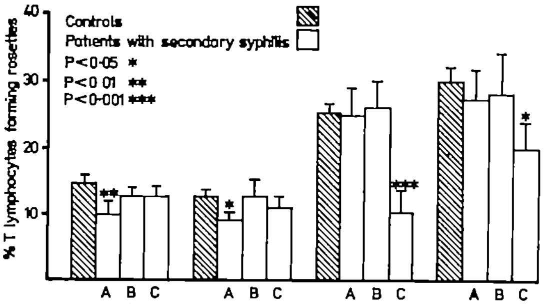


FIG 2 *The percentage of Tγ and Tμ rosettes in patients with secondary syphilis before treatment (A, n = 9), one month after (B, n = 11), and two months after treatment (C, n = 12) in T cells cultured for 24 hours and 48 hours compared with controls (n = 84). Results represent mean ± SEM (Student's t test).*

percentage of Tμ lymphocytes was normal whereas that of Tγ cells was significantly reduced (fig 2).

The reduced percentage of Tγ cells during the secondary stage was investigated in vitro by adding serum from each of three patients with secondary syphilis to T lymphocytes from three healthy donors. Complement was not inactivated by heat. The expression of the Fc-IgG receptor on the T lymphocytes was significantly reduced without selective killing of Tγ cells ($t_2=6\cdot48$, $P<0\cdot025$). When the immunoglobulin in serum was removed through ammonium sulphate precipitation this effect disappeared (table III). No reduction of the expression of the Fc-IgM receptor was observed in similar experiments using serum from a patient with primary syphilis (results are not shown).

Discussion

The importance of cell-mediated immunity (CMI) in syphilis is suggested by T-lymphocyte depletion in the paracortical areas of lymph nodes and the periarteriolar area of the spleen in children with fatal congenital syphilis.[9, 10] In rabbits, the infection takes a severe course if T lymphocytes are destroyed by cyclophosphamide treatment.[11] In patients with syphilis evidence has been found for a depression in CMI, which involves cutaneous reactivity,[1] low T-cell numbers,[6] low in-vitro reactivity to *Treponema pallidum,*[1, 3-5] and immunosuppressive factors in serum.[1, 2]

This study demonstrates that the total number of T lymphocytes are reduced in patients with early syphilis. The T lymphopenia may be due to an unequal distribution of lymphocytes in the lymphoid system, although a depletion of lymphocytes is also seen in histological examinations of enlarged lymph nodes during early syphilis.[12] Another possibility is that cytotoxic antibodies to T lymphocytes may occur, although lymphocytotoxic antibodies were shown to be active only at 15°C.[13] Also, the T lymphopenia disappears rapidly after treatment and the serum assays do not show any enhanced cell death. A third possibility is that *T pallidum* itself is directly toxic to T lymphocytes.

It has been proposed that T lymphocytes with Fc receptors may function as helper (Tμ) or suppressor (Tγ) cells.[14] In primary syphilis the Tμ-cell concentration was low, consistent with compartmentalisation of helper cells—that is, an unequal distribution of lymphocytes in the body during an immune response.[15] Compartmentalisation of helper cells occurs during early antibody production.[16, 17] The Tγ-cell concentration was increased, perhaps as a result of suppressor-cell activation.[18]

In patients with secondary syphilis the Tμ-cell concentration was normal until two months after treatment when a fall occurred. The reason for this fall is obscure. During active secondary syphilis the Tγ-cell concentration was significantly reduced. Explanations for this may be similar to those for the T lymphopenia. Furthermore, during secondary syphilis immune complex formation takes place,[19] and it is well known that immune complexes interact in vitro with Tγ cells leading to an irreversible loss of the Fc receptor for IgG.[20] This mechanism may explain the reduction of the Tγ cells in peripheral blood as indicated by the results from the serum assays (table III). No similar mechanism seemed to operate in primary syphilis.

It has been found that Tγ cells activated by pokeweed mitogen and immune complexes release immunosuppressive factors.[21] *T pallidum* may give an in-vivo stimulation, which in conjunction with immune complexes stimulates Tγ cells to release immunosuppressive factors. This may explain the

TABLE III *Effect of secondary syphilitic serum on homologous T lymphocyte subpopulations in three controls*

		Percentage of T lymphocyte subpopulations in controls:		
Donor serum (40%)	*Subpopulations*	*Control 1*	*Control 2*	*Control 3*
Autologous serum	Tγ	9·0	15·0	13·0
	Tμ	31·5	53·0	40·5
Autologous serum without Ig	Tγ	9·5	18·0	16·0
	Tμ	29·5	36·5	39·0
Secondary syphilitic serum	Tγ*	3·0	4·0	4·0
	Tμ	33·0	50·5	43·5
Secondary syphilitic serum without Ig	Tγ	8·5	17·0	14·0
	Tμ	33·0	37·0	43·0
Controls without serum	Tγ	11·0	20·0	17·5
	Tμ	33·0	36·0	40·5

*Significant reduction (78%; $t_2=6\cdot48$, $P<0\cdot025$)

reduced cutaneous reactivity in syphilitic patients[1] and the depressed in-vitro functions of lymphocytes from peripheral blood.[1 3-5]

Reduction of the $T\gamma$-cell concentration has been observed in various autoimmune diseases and in other diseases with increased antibody production.[22-28] The increased antibody production may be caused by direct polyclonal B-cell activation or indirectly by a defective suppressor-cell mechanism, which may be a primary disorder or secondary to the occurrence of lymphocytotoxic antibodies or immune complexes. In systemic lupus erythematosus (SLE) the suppressor-cell defect is mainly a quantitative rather than a qualitative disorder.[29] Immunosuppressive factors, which depress non-specific suppressor-cell activity, have also been found in SLE.[30 31]

The changes observed in the subpopulations of T lymphocytes occurred during an infectious disease in young subjects who were not suspected of having any immune deficiencies. These changes are similar to those found in various diseases with an imbalance between an increased B-cell activity and signs of reduced immunoregulatory T cells.[22-28] Our findings seem to show that the changes in immunoregulatory T cells may be secondary to a strong immune reaction —that is, treponemal infection.

This work was supported by the Danish Medical Research Council, grants Nos 512-15133, 512-20160, and 512-20207. We wish to thank Mrs A Jespersen and J Schjøth for technical assistance and Dr K Thestrup-Pedersen for his constant encouragement and for reviewing the manuscript.

References

1. From E, Thestrup-Pedersen K, Thulin H. Reactivity of lymphocytes from patients with syphilis towards *T pallidum* antigen in the leucocyte migration and lymphocyte transformation tests. *Br J Vener Dis* 1976;**52**:224-9.
2. Levene GM, Turk JI, Wright DJM, Grimble AGS. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. *Lancet* 1969;ii:246-7.
3. Fulford KWM, Brostroff J. Leucocyte migration and cell-mediated immunity in syphilis. *Br J Vener Dis* 1972;**48**:483-8.
4. Pavia CS, Baseman JB, Folds JD. Selective response of lymphocytes from *Treponema pallidum*-infected rabbits to mitogens and *Treponema reiteri. Infect Immun* 1977;**15**:417-22.
5. Pavia CS, Folds JD, Baseman JB. Selective in-vitro response of thymus-derived lymphocytes from *Treponema pallidum*-infected rabbits. *Infect Immun* 1977;**18**:603-11.
6. Bos JD, Hamerlinck F, Cormane RH. T lymphoid cells in primary syphilis. Quantitative studies. *Br J Vener Dis* 1980;**56**:74-6.
7. Kaplan ME, Clark C. An improved rosetting assay for the detection of human T lymphocytes. *J Immunol Methods* 1974;**5**:131-5.
8. Gupta S, Good RA. Subpopulations of human T lymphocytes I. Studies in immunodeficient patients. *Clin Exp Immunol* 1977;**30**:222-8.
9. Levene GM, Wright DJM, Turk JL. Cell-mediated immunity and lymphocyte transformation in syphilis. *Proc R Soc Med* 1971;**64**:426-8.
10. Wright DJM, Grimble AS. Why is the infectious stage of syphilis prolonged? *Br J Vener Dis* 1974;**50**:45-9.
11. Pacha J, Metzger M, Smogór W, Michalska E, Podwinska J, Ruczkowska J. Effect of immunosuppressive agents on the course of experimental syphilis in rabbits. *Arch Immunol Ther Ex (Warsz)* 1979;**27**:45-51.
12. Turner DR, Wright DJM. Lymphadenopathy in early syphilis. *J Pathol* 1973;**110**:305-8.
13. de Jong NHL, Koehorst JAM, van der Sluis JJ, Boer AM. Autolymphocytotoxins in syphilis. *Br J Vener Dis* 1980;**56**:297-301.
14. Moretta L, Webb SR, Grossi CE, Lydyard PM, Cooper MD. Functional analysis of two human T-cell subpopulations, help and suppression of B-cell responses by T cells bearing receptors for IgM and IgG. *J Exp Med* 1977;**146**:184-200.
15. Schlossman SF, Levin HA, Rocklin RE, David JR. The compartmentalization of antigen-reactive lymphocytes in desensitized guinea pigs. *J Exp Med* 1971, **134**:741-50.
16. Lagrange PH, Mackaness GB, Miller TE. Influence of dose and route of antigen injection on the immunological induction of T cells. *J Exp Med* 1974;**139**:528-42.
17. Hahn H, Kaufmann SHE, Falkenberg F, Chahinin M, Horn W. Peritoneal exudate T lymphocytes with specificity to sheep red blood cells. II Inflammatory helper T cells and effector T cells in mice with delayed-type hypersensivity and in suppressed mice. *Immunology* 1979;**38**:51-5.
18. Ramshaw IA, Bretscher PA, Parish CR. Regulation of the immune response. I Suppression of delayed type hypersensivity by T cells from mice expressing humoral immunity. *Europ J Immunol* 1979;**6**:674-9.
19. Sølling J, Sølling K, Jakobsen KU, From E. Circulating immune complexes in syphilis. *Acta Derm Venereol (Stockh)* 1978;**58**:263-7.
20. Pichler WJ Lum L, Border S. Fc receptors on human T lymphocytes. I Transition of $T\gamma$ to $T\mu$ cells. *J Immunol* 1978;**121**:1540-8.
21. Moretta L, Mingari MC, Moretta A, Cooper MD. Human T-lymphocyte subpopulations: studies of the mechanism by which T cells bearing Fc receptors for IgG suppress T-dependent B cell differentiation induced by PWM. *J Immunol* 1979;**122**:984-90.
22. Fauci AS, Steinberg AD, Haynes BF, Whalen G. Immunoregulatory abberrations in systemic lupus erythematosus. *J Immunol* 1978;**121**:1473-9.
23. Alarcón-Segovia D, Ruiz-Argüelles A. Decreased circulation thymus-derived cells with receptors for the Fc portion of immunoglobulin G in systemic lupus erythematosus. *J Clin Invest* 1978;**62**:1390-4.
24. Reinherz EL, Rubinstein A, Geha RS, Strelkauskas AJ, Rosen FS, Schlossman SF. Abnormalities of immunoregulatory T cells in disorders of immune function. *N Engl J Med* 1979;**301**:1018-22.
25. Sandilans GP, Galbraith I, Reid FM, Mills PR, MacSween RMN. Immune-complex inhibition of lymphocyte Fc receptors in primary biliary cirrhosis. A possible immunomodulatory mechanism. *Lancet* 1980;ii:9-13.
26. Reinherz EL, Weiner HL, Hauser SL, Cohen JA, Distaso JA, Schlossman SF. Loss of suppressor T cells in active multiple sclerosis. Analysis with monoclonal antibodies. *N Engl J Med* 1980;**303**:125-9.
27. Bach M-A, Phan-Dinh-Tuy F, Tournier E, Chatenoud L, Bach J-F. Deficit of suppressor T cells in active multiple sclerosis. *Lancet* 1980;ii:1221-3.
28. Jensen JR, Cramers M, Thestrup-Pedersen K. Subpopulations of T lymphocytes and non-specific suppressor-cell activity in patients with atopic dermatitis. *Clin Exp Immunol* 1981;**45**:118-23.
29. Moretta M, Mingari MC, Santoli, D, Perlman P, Moretta L. Human T-lymphocyte subpopulations: alterations in systemic lupus erythematosus. *Scand J Immunol* 1979;**10**:223-8.
30. Sagawa A, Abdou NI. Suppressor-cell antibody in systemic lupus erythematosus. Possible mechanism for suppressor-cell dysfunction. *J Clin Invest* 1979;**63**:536-9.
31. Sakane T, Steinberg AD, Reeves JP, Green I. Studies of immune functions of patients with systemic lupus erythematosus. Complement-dependent immunoglobulin M anti-thymus-derived cell antibodies preferentially inactivate suppressor cells. *J Clin Invest* 1979;**63**:954-65.

Br J Vener Dis 1982;**58**:23-8

In-vitro activity of 21 antimicrobial agents against Neisseria gonorrhoeae in Brussels

B GORDTS, R VANHOOF, J M HUBRECHTS, R DIERICKX, H COIGNAU, AND J P BUTZLER

From the Microbiology Laboratory, University Hospital (St Pieters), Brussels, Belgium

SUMMARY The minimum inhibitory concentrations (MIC) of 21 antimicrobial agents was measured for 80 strains of *Neisseria gonorrhoeae* isolated in Brussels in 1978. Bimodal distributions were found for penicillin G, ampicillin, amoxycillin, carbenicillin, and cephalexin. Of the strains, 17·5% were relatively resistant to penicillin G (MIC >0·08 μg/ml), 27·5% to ampicillin (MIC >0·16 μg/ml), 23·8% to amoxycillin, and 43·3% to carbenicillin. Cefotaxime was the most active antibiotic, with MICs in the nanogram range; 3·8% and 5% of the strains were relatively resistant to cephaloridine and cephalexin respectively, but no strains were resistant to cefazolin, cefuroxime, or cefotaxime. Resistance to tetracycline, doxycycline, minocycline, erythromycin, and spiramycin (MIC >1 μg/ml) was found in 6·3%, 2·5%, 2·5%, 5%, and 51·3% of the strains respectively.

A very good correlation was present between chloramphenicol and thiamphenicol, with 16·3% and 10% of relatively resistant strains respectively. Only two isolates showed an MIC >1·25 μg/ml for rifampicin, and 10% of the strains needed ⩾12μg/ml of spectinomycin for complete inhibition of growth. A very high synergy was found for the 20 : 1 combination of sulphamethoxazole and trimethoprim, with only one isolate resistant to this combination. None of the strains tested produced β-lactamase.

Introduction

Many studies have been carried out in Western Europe over the last 20 years to test the in-vitro sensitivity of *Neisseria gonorrhoeae* to various antimicrobial agents. A definite progression towards decreased sensitivity to various antibiotics has been noticed.[1] Greater importance has been attached to the regional differences in the sensitivity patterns of gonococci.[2 3] It seems important, therefore, to continue screening the sensitivity of gonococci in different parts of the world. This paper describes quantitative sensitivity determinations of 80 unselected strains of *N gonorrhoeae* isolated before treatment in the venereal disease clinic at the St Pieters Hospital in Brussels from 1 January to 31 December 1978. The results are compared with those of other workers in other regions and with those of a similar study in the same hospital in 1976.[4]

Address for reprints: Dr B Gordts, Microbiology Laboratory, University Hospital (St Pieters), Hoogstraat 322, B-1000 Brussels, Belgium

Accepted for publication 1 September 1981

Materials and methods

ISOLATION OF GONOCOCCAL STRAINS

Eighty strains of *N gonorrhoeae* were isolated from male and female patients attending the venereal diseases clinic at the St Pieters Hospital in Brussels during 1978. Immediate diagnosis was made by the examination of Gram-stained smears. The strains were cultured on selective Thayer-Martin medium and incubated at 37°C in 10% CO_2 for 48 hours. The organisms were identified by colonial morphology, Gram-staining, oxidase activity, and sugar fermentation. The purified cultures were suspended in sterile horse serum and stored in liquid nitrogen until investigated further.

SENSITIVITY TESTING

Antibiotic sensitivity testing was performed by a dilution method in liquid medium. The strains were cultured on a medium containing brain-heart infusion (BHI) broth (Difco 0037-01) and 1·5% agar enriched with 10% defibrinated horse blood (Institut Pasteur, Brussels) and 1% sterile GC supplement (Oxoid SR 56). The plates were incubated in 10%

CO_2 for 48 hours. The resulting colonies were then suspended in a Mueller-Hinton broth to give 3×10^8 colony-forming units (cfu)/ml (McFarland No 1).

The medium used to test the sensitivity of the antimicrobial agents was Mueller-Hinton broth to which was added 1% Polyvitex (Bio-Merieux 5-5651), 5% of a haemin solution (600 μg/ml) (BDH-Biochemicals 24011), and 2% sterile horse serum. To test the sensitivity to the sulphonamides, 5% haemolysed horse blood (Institut Pasteur, Brussels) was used instead of haemin in the medium. The medium was dispensed in microtitre plates (Dynatech MA1501/N), together with two-fold dilutions of the antibiotics, by a Dynatech 96 Channel Dispenser. The test plates were inoculated with the suspension by a MIC 2000 Dynatech Inoculator which places 1 μl of the suspension in every test cup of 100 μl, giving a final concentration of 3×10^6 cfu/ml. In every test plate, one row was left without any antibiotic. Two reference strains of *N gonorrhoeae* (Reyn 181, Reyn 183) with known sensitivity patterns were tested simultaneously in each run. The plates were incubated in 10% CO_2 and examined after 48 hours in a Dynatech Viewbox. The lowest antibiotic concentration that inhibited bacterial growth completely, or almost completely, was regarded as the minimum inhibitory concentration (MIC).

ANTIMICROBIAL AGENTS

The following antimicrobial agents were tested: penicillin G (Continental Pharma), ampicillin (Bristol), amoxycillin and carbenicillin (Beecham Laboratories), cephaloridine, cephalexin, and cefazolin (Eli Lilly Laboratories), cefuroxime (Glaxo), cefotaxime (Hoechst), erythromycin (Abbott Laboratories), spiramycin (Specia), tetracycline HCL (Certa), doxycycline (Pfizer), minocycline (Lederle), chloramphenicol and rifampicin (Lepetit), thiamphenicol (Zambon), spectinomycin (Upjohn), trimethoprim and sulphamethoxazole (Roche SA) and a combination of sulphamethoxazole and trimethoprim in a 20 : 1 ratio.

Screening for β-lactamase production was performed by the chromogenic cephalosporin test (Glaxo compound 87/312).

Results

The distribution of the MICs for the 21 antimicrobial agents tested is shown in tables I and II. Bimodal distributions were found with penicillin G, ampicillin, amoxycillin, carbenicillin, and cephalexin (figure). Tests for β-lactamase production gave negative results for all the 80 strains.

Penicillin G showed a first peak at 0·048 μg/ml and a second at 0·012 μg/ml. We defined relative resistance (RR) as an MIC >0·08 μg/ml; 17·5% of the strains fell into this group, and 6·3% of the strains showed "high level resistance" to penicillin G (MIC >0·39 μg/ml). For ampicillin the two peaks occurred at 0·097 and 0·012-0·006 μg/ml and 27·5% of strains were considered to be relatively resistant (MIC >0·16 μg/ml).

The peaks for amoxycillin were at 0·195-0·097 μg/ml and 0·012-0·006 μg/ml; 23·8% of the strains were RR. Carbenicillin showed peaks at 0·195 μg/ml and 0·006 μg/ml; 43·3% were RR. A bimodal distribution was also found for cephalexin, with a first modus at 0·195-0·39 μg/ml and a second at 1·56 μg/ml. For the second modus strains of cephalexin only five out of 23 were also RR to penicillin G, while 10 strains were RR to ampicillin, nine to amoxycillin, and 15 to carbenicillin.

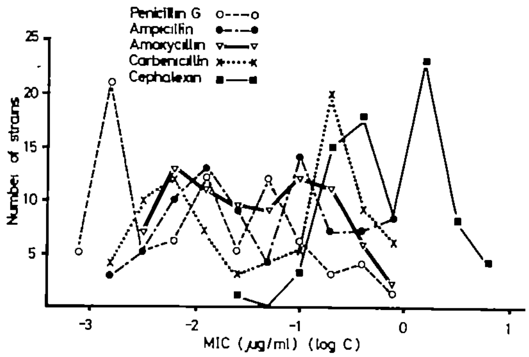


FIGURE *Distribution of MICs for penicillin G, ampicillin, amoxycillin, carbenicillin, and cephalexin.*

For the cephalosporins 3·8% of the strains were RR to cephaloridine (MIC >3·12 μg/ml), while 5% were RR to cephalexin. All were sensitive to cefazolin, cefuroxime, and cefotaxime. Among the tetracyclines, doxycycline and minocycline showed a comparable activity against the isolates. For tetracycline, doxycycline, and minocycline respectively 6·25, 2·5, and 2·5% of the isolates were RR (MIC >1μg/ml). Two isolates that were RR to tetracycline were also RR to penicillin G, while among the 14 strains that were RR to penicillin G only two were also RR to tetracycline.

Both chloramphenicol and thiamphenicol showed a peak at 0·195 μg/ml; 16·3% of the strains were RR to chloramphenicol and 10% to thiamphenicol. Erythromycin was more active than spiramycin, with only 5% being RR to it, while 51·3% were RR to spiramycin. For rifampicin two strains required 50 μg/ml or more to inhibit growth, but all the others (97·5%) were sensitive.

TABLE I *Distribution of MICs of 20 antimicrobial agents against 80 strains of gonococci*

	Minimum inhibitory concentrations (μg/ml)																			
Antimicrobial agents	*0·00037*	*0·00075*	*0·0015*	*0·003*	*0·006*	*0·012*	*0·024*	*0·048*	*0·097*	*0·195*	*0·39*	*0·78*	*1·56*	*3·12*	*6·25*	*12·5*	*25*	*50*	*100*	*>100*
Penicillin G		5	21	5	6	12	5	12	6	3	4	1								
Ampicillin			3	5	10	13	9	4	14	7	7	8								
Amoxycillin				7	13	11	9	9	12	11	6	2								
Carbenicillin			4	10	12	7	3	4	5	20	9	6								
Cephaloridine								4	6	10	17	25	11	4	1	2				
Cephalexin							1		3	15	18	8	23	8	4					
Cefazolin					4	2	3	8	15	13	22	6	7							
Cefuroxime		1	19	12	16	14	6	2	3	2	5									
Cefotaxime	28	22	18	9				1		1	1									
Erythromycin						1	6	4	20	23	13	9	2	2						
Spiramycin							1		3	6	6	23	16	16	5	3				
Tetracycline						3	11	8	14	17	11	11	3	2						
Doxycycline						5	13	14	18	15	8	5		1	1					
Minocycline					4	6	9	10	17	20	9	3	2							
Chloramphenicol							1	2	19	23	19	3	5	4	2	2				
Thiamphenicol							1		11	42	9	9	4	2	1	1				
Spectinomycin									2	4	2	5	7	25	27	5	2	1		
Rifampicin			1		12	8	19	21	12	4		1						1		1
	0·24	*0·48*	*0·97*	*1·95*	*3·9*	*7·8*	*15·6*	*31·2*	*62·5*	*125*	*250*									
Sulfamethoxazole		2	4	16	19	26	8	3	2											
	0·024	*0·048*	*0·097*	*0·195*	*0·39*	*0·78*	*1·56*	*3·12*	*6·25*	*12·5*	*25*	*50*	*100*	*>100*						
Trimethoprim						1	1		4	10	23	19	21	1						

TABLE II *Minimum inhibitory concentration of 20 : 1 combination of sulphamethoxazole and trimethoprim*

	Sulphamethoxazole (µg/ml)								
Trimethoprim (µ/ml)	*0·12*	*0·24*	*0·48*	*0·97*	*1·95*	*3·9*	*7·8*	*15·6*	*31·2*
0·006	1								
0·012		1							
0·024			1						
0·048				3					
0·097					17				
0·195						31			
0·39							22		
0·78								3	
1·56									1

Ten per cent of the strains needed 12 µg/ml of spectinomycin for complete inhibition of growth. With the sulphonamides 51·3% were RR to trimethoprim (MIC >25 µg/ml) and 6·3% to sulphamethoxazole (MIC >15·6 µg/ml). The 20 : 1 combination of sulphamethoxazole and trimethoprim resulted in a significant reduction in the MICs.

The number of strains that were cross-resistant with penicillin G are shown in table III. We found no multiresistant strains. The contingency coefficient C, which measures the extent of correlation between two sets of attributes and is computed from 2 × 2 contingency table for all pairs of antimicrobial agents, is given in table IV. The significance of the C factor is tested by the χ^2 test.[5a]

A very good correlation was found between the different penicillins and the cephalosporins. Only penicillin G and ampicillin showed a correlation with cephalexin. The penicillins also had a poor correlation with the tetracyclines, except for amoxycillin, which showed a slightly better correlation with the tetracyclines. No correlation was found between the different cephalosporins, except for a slightly better correlation between cephalexin and cephaloridine.

TABLE III *Strains relatively resistant to penicillin G which showed cross-resistance to other antibacterial agents**

	Strains	
Antimicrobial agents	*No*	*%*
Ampicillin	9	64·3
Amoxycillin	8	57·1
Carbenicillin	11	78·6
Cephaloridine	1	7·1
Cephalexin	3	21·4
Cefazolin	0	0
Cefuroxime	0	0
Cefotaxime	0	0
Erythromycin	4	28·6
Spiramycin	13	92·9
Tetracycline	2	14·3
Doxycycline	1	7·1
Minocycline	0	0
Chloramphenicol	6	42·9
Thiamphenicol	4	28·6
Spectinomycin	5	35·7
Rifampicin	0	0
Trimethoprim	5	35·7
Sulphamethoxazole	1	7·1
Co-trimoxazole	0	0

*14 strains relatively resistant to penicillin G (MIC >0·08 µg/ml)

Erythromycin showed a poor correlation with spiramycin, and no correlation with the different tetracyclines. Tetracycline showed a good correlation with doxycycline and minocycline, but there was no correlation between the two latter antibiotics. The correlation between chloramphenicol and thiamphenicol was excellent. Rifampicin showed no correlation with any other antibiotic. This was also found for sulphamethoxazole; a correlation was only found between co-trimoxazole and sulphamethoxazole but not between the sulphonamides and any other of the antimicrobial agents tested.

Discussion

The results for penicillin G (17·5% RR) show that there has been a marked decrease in the proportion of RR strains of *N gonorrhoeae* since the study of Vanhoof *et al,*[4] in which 51% of the strains were RR. Meheus *et al*[5b] found a similar result as in the present study. A decrease in RR strains has already been described by Olsen and Lomholt[6] in Greenland and by Seth and Wilkinson[7] in London. For ampicillin, we found that 27·5% of the strains were RR. This is a lower percentage than that reported by Meheus *et al,*[5b] and much lower than in the former study of Vanhoof *et al* in Brussels.[4]

The other penicillins (amoxycillin and carbenicillin) also showed a lower proportion of RR strains than reported in the former Belgian studies. For cephalexin, a bimodal distribution was obvious, but only 17% of the second modus strains were also RR to penicillin G in contrast to the former study of Vanhoof *et al,*[4] in which all second modus strains of cephalexin were found to be RR to penicillin G.

In general, very good sensitivity was found for the new cephalosporins, with no RR strains at all and all MICs >0·78 µg/ml. Cefotaxime was the most active of all the antibiotics tested in this study. We found that 6·3% of the strains were RR to tetracycline,

TABLE IV *Contingency coefficient C computed from a 2 × 2 contingency table for all pairs of antimicrobial agents (significance by χ^2 test)*

	PEN	*AMP*	*AMX*	*CAR*	*CRD*	*CLX*	*CZL*	*CFX*	*CTX*	*ERY*	*SPI*	*TET*	*DOX*	*MIN*	*CHL*	*THI*	*SPE*	*RIF*	*TMP*	*SMZ*	*SXT*
PEN																					
AMP	0·47‡																				
AMX	0·34†	0·41‡																			
CAR	0·30†	0·35†	0·46†																		
CRD	0·08§	0·12§	0·20§	0·09§																	
CLX	0·33†	0·24*	0·14§	0·14§	0·25*																
CZL	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§															
CFX	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§														
CTX	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§	0·00§													
ERY	0·32†	0·11§	0·01§	0·25*	0·05§	0·20§	0·00§	0·00§	0·00§												
SPI	0·36‡	0·02§	0·19§	0·15§	0·06§	0·22*	0·00§	0·00§	0·00§	0·22*											
TET	0·15§	0·18§	0·32†	0·19§	0·21§	0·06§	0·00§	0·00§	0·00§	0·18§	0·25*										
DOX	0·14§	0·10§	0·28*	0·18§	0·20§	0·10§	0·00§	0·00§	0·00§	0·04§	0·15§	0·29†									
MIN	0·07§	0·08§	0·28*	0·18§	0·20§	0·10§	0·00§	0·00§	0·00§	0·04§	0·15§	0·29†	0·04§								
CHL	0·32†	0·11§	0·07§	0·28†	0·24*	0·34*	0·00§	0·00§	0·00§	0·34†	0·22*	0·16§	0·34†	0·34†							
THI	0·27*	0·26*	0·11§	0·13§	0·35‡	0·45‡	0·00§	0·00§	0·00§	0·29†	0·16§	0·09§	0·21§	0·21§	0·60‡						
SPE	0·37‡	0·26*	0·11§	0·13§	0·15§	0·29†	0·00§	0·00§	0·00§	0·29†	0·08§	0·09§	0·21§	0·03§	0·39‡	0·40‡					
RIF	0·07§	0·10§	0·09§	0·02§	0·03§	0·04§	0·00§	0·00§	0·00§	0·04§	0·16§	0·04§	0·03§	0·03§	0·07§	0·05§	0·05§				
TMP	0·14§	0·13§	0·10§	0·15§	0·04*	0·12§	0·00§	0·00§	0·00§	0·12§	0·00§	0·06§	0·00§	0·00§	0·07§	0·17§	0·01§	0·16§			
SMZ	0·02§	0·04§	0·01§	0·08§	0·05§	0·18§	0·00§	0·00§	0·00§	0·06§	0·06§	0·07§	0·04§	0·04§	0·03§	0·09§	0·09§	0·04§	0·16§		
SXT	0·05§	0·18§	0·06§	0·13§	0·02§	0·03§	0·00§	0·00§	0·00§	0·03§	0·11§	0·03§	0·02§	0·02§	0·05§	0·04§	0·04§	0·02§	0·11§	0·40‡	

* $6·635 > \chi^2 > 3·841$, $0·01 < P < 0·05$
† $10·832 > \chi^2 > 6·635$, $0·001 < P < 0·01$
‡ $\chi^2 > 10·832$, $P < 0·001$
§ $3·841 > \chi^2$, $0·05 < P$

PEN = penicillin G; AMP = ampicillin; AMX = amoxycillin; CAR = carbenicillin; CRD = cephaloridine; CLX = cephalexin; CZL = cefazolin; CFX = cefuroxime; CTX = cefotaxime; ERY = erythromycin; SPI = spiramycin; TET = tetracycline; DOX = doxycycline; MIN = minocycline; CHL = chloramphenicol; THI = thiamphenicol; SPE = spectinomycin; RIF = rifampicin; TMP = trimethoprim; SMZ = sulphamethoxazole; SXT = co-trimoxazole.

which is very low compared with the studies of Stolz and Zwart,[2] Bergogne-Berezin,[1] Meheus *et al*,[5b] and Jaffe *et al.*[3] Doxycycline and minocycline showed comparable activity. Five per cent of the strains seemed to be RR to erythromycin, which is greater than that found by Meheus.[4] Robson and Salit,[8] and Givan and Keyl.[9] Erythromycin showed a much higher activity than spiramycin. Ten per cent of the strains needed ⩾12 μg/ml of spectinomycin to be inhibited. This seems to be high in comparison with other studies. Only about 2·5% of the strains were RR to rifampicin, and this agrees well with former results. Once again, the synergy between sulphamethoxazole and trimethoprim was noticed. Only one strain showed relative resistance to the 20:1 combination.

We did not find any β-lactamase-producing gonococcal strains in our population; these strains are extremely rare in Belgium.[10]

References

1. Bergogne-Berezin E, Zechovsky N, Siboulet A. Sensibilité actuelle de *Neisseria gonorrhoeae*. Etude de 100 souches dans la région Parisienne. *Sem Hop Ther* 1975;**51**:81-90.
2. Stolz E, Zwart HGF, Michel MF. Activity of eight antimicrobial agents in vitro against *Neisseria gonorrhoeae*. *Br J Vener Dis*, 1975;**51**:257-64.
3. Jaffe HW, Biddle JW, Thornsberry C, *et al.* National gonorrhoea therapy monitoring study. In-vitro antibiotic susceptibility and its correlation with treatment results. *N Engl J Med*, 1976;**294**:5-9.
4. Vanhoof R, Vanderlinden MP, Hubrechts JM, Butzler JP, Yourassowsky E. In-vitro activity of antimicrobial agents against *Neisseria gonorrhoeae* in Brussels. *Br J Vener Dis* 1976;**52**:197-8.

5a. Siegel S. *Non-parametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill-Kogakusha, 1956;196-202.

5b. Meheus A, Piot P, Pattyn S, Van Dyck E, Vanden Berghe D. Activity in vitro of 10 antimicrobial agents against *Neisseria gonorrhoeae*. A study of the correlation between the sensitivities. *Br J Vener Dis* 1976;**52**:329-32.

6. Olson GA, Lomholt G. Gonorrhoea treated by a combination of probenecid and sodium penicillin G. *Br J Vener Dis* 1969;**45**:144.
7. Wilkinson AE. The sensitivity of gonococci to penicillin. *J Antimicrob Chemother* 1977;**3**:197-8.
8. Robson HG, Salit IE. Susceptibility of *Neisseria gonorrhoeae* to seven antibiotics in vitro. *Can Med Assoc J* 1972;**107**:959-62.
9. Givan KF, Keyl A. Antibiotic sensitivities of *Neisseria gonorrhoeae* in the Toronto area. *Can Med Assoc J* 1974:**111**:44-6.
10. Piot P. Resistant gonococcus from the Ivory coast. *Lancet* 1977;**i**:857.

Br J Vener Dis 1982; **58**: 29-31

Penicillinase-producing gonococcal strains in Zambia*

Observations on treatment failures

ATTILI V RATNAM, MOHMEDALI I PATEL, RAPHAEL C MULENGA, AND SUBHASH K HIRA

From the Dermatovenereology Division, Department of Medicine, University Teaching Hospital, Lusaka, Zambia

SUMMARY Penicillinase-producing strains of *Neisseria gonorrhoeae* (PPNG) were detected in nine out of 27 (3·2%) treatment failures in 310 cases of acute gonococcal urethritis in men in Lusaka, Zambia. Minimum inhibitory concentrations of penicillin for 17·2% of 233 gonococcal isolates were $\leqslant$0·05 μg/ml, for 38·2% between 0·125 and 0·25 μg/ml, and for 46·6% $\geqslant$0·5 μg/ml.

At present the prevalence of PPNG in African countries is not known but is likely to increase rapidly unless simplified control schemes are adopted within the existing primary health care programmes. Endemic pockets of PPNG in a few countries can threaten worldwide efforts to control gonorrhoea.

Introduction

Although penicillin is still the drug of choice for the treatment of gonorrhoea, continued surveillance of the susceptibility of gonococci to penicillin has become mandatory because of increasing drug resistance. The emergence of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) in 1976, and their increasing prevalence in many parts of the world,[1] has been a further serious challenge to effective antibiotic treatment. It has been observed that PPNG not only survive high doses of various penicillins but are also less susceptible to many other antibiotics.[2 3] Reports from many countries[4-7] indicate a higher prevalence of gonorrhoea in Africa than elsewhere and that gonococcal strains which are less susceptible to penicillin are also more prevalent.[8-12] Zambia is no exception, high incidences of gonorrhoea have been reported among patients attending the sexually transmitted diseases clinic in Lusaka,[13] in pregnant women,[14] and in patients with pelvic inflammatory disease.[15]

In this paper we have reviewed the minimum inhibitory concentrations (MICs) of penicillin to 233 gonococcal strains and the clinical and laboratory aspects of 27 cases of treatment failure.

Patients and methods

DIAGNOSIS

Between August 1980 and February 1981 gonococcal infection was diagnosed in 574 men. The presence of typical Gram-negative intracellular diplococci in urethral smears was considered adequate for diagnosis. Specimens for culture were also taken from 420 men, and gonococci were isolated in 391.

TREATMENT

Of these 574 men, 265 received aqueous procaine penicillin (APPG) 4·8 megaunits by injection with probenecid 1g orally, 185 amoxycillin 3 g in a single oral dose, and 124 an injection of spectinomycin 2 g.

FOLLOW UP

After treatment 485 men had test-of-cure cultures performed after 48 hours, and 351 attended for a second examination after two weeks. Treatment failure was diagnosed if gonococci reappeared either in Gram-stained urethral smears or in cultures during the two-week follow-up period in the absence of further sexual contact. By these criteria treatment failure was suspected in 27 cases, of which 23 were confirmed by positive culture results. In all the 27 cases the initial drug regimen was repeated, and

---

*A slightly modified version of this paper was presented at the Second IUVDT Conference, Dakar, 4-9 May 1981

Address for reprints: Dr A V Ratnam, Dermatovenereology Division, University Teaching Hospital, Private Bag RW 50001, Lusaka, Zambia

Accepted for publication 24 July 1981

patients were reassessed. If the second treatment also failed to effect cure, patients were retreated with spectinomycin 2 g.

BACTERIOLOGY

Thayer-Martin medium, containing vancomycin, colistin, and nystatin, was used for primary isolation of gonococci. Urethral discharge or a scraping obtained with a sterile 3-mm platinum loop was directly plated in the clinic and incubated in a candle-extinction jar at 37°C for 24 to 48 hours. Positive cultures were identified by typical colonial appearance, oxidase reaction, and examination by Gram's stain.

MINIMUM INHIBITORY CONCENTRATIONS

An agar plate dilution technique was used to determine the MICs of penicillin. Saline suspension of the test strains and a control strain (*Staphylococcus aureus* ATCC 25923), corresponding to a McFarland No 5 tube, were prepared and a 3-mm loopful of these suspensions used to inoculate chocolate agar plates enriched with Isovitalex, containing doubling dilutions of benzyl penicillin from 2·0 to 0·0125 μg/ml, and a control plate without penicillin. (This standardisation of the inoculum was not performed during the earlier part of the study.) The inoculated plates were inspected for growth after 24 hours' incubation, and the MIC was taken as the lowest concentration at which no visible growth occurred.

PENICILLINASE PRODUCTION

A total of 310 gonococcal isolates were also screened for penicillinase production by a rapid iodometric method.[1] Penicillinase-positive and penicillinase-negative reference strains (CDC-76-073389, F-18) were tested with each batch. Ten PPNG strains were identified (one from a patient successfully treated with spectinomycin) and their antibiotic sensitivities also tested with discs of penicillin 6 μg, ampicillin 25 μg, amoxycillin 25 μg, co-trimoxazole 25 μg and tetracycline 30 μg. Isolates with an inhibitory zone of more than 10mm in diameter were considered sensitive.

Results

SENSITIVITIES

MICs of penicillin for 233 gonococcal strains, including those from 23 of 27 treatment failures, are shown in the table. MICs of only 17·2% of strains were within the sensitive range (≤0·05 μg/ml). Intermediate sensitivity (0·125-0·25 μg/ml) was observed for 38·2% of strains, and 46·6% showed a greater degree of resistance (≥0·5 μg/ml). MICs of five strains from treatment failures were in the intermediate sensitivity range, and 18 strains, including nine PPNG, had MICs of >0·5 μg/ml. By the disc test all the 10 PPNG strains were resistant to 6 μg of penicillin and to 25 μg each of ampicillin and amoxycillin.

TABLE *Penicillin sensitivities of 233 gonococcal isolates*

MICs of penicillin (μg/ml)	*No (%) of strains*	*No of treatment failures*
0·0125-0·05	40 (17·2)	0
0·125-0·25	89 (38·2)	5
0·5-2·0	104 (44·6)	18*

* Includes 9 PPNG Strains

Three strains were sensitive to tetracycline and only one was sensitive to co-trimoxazole.

TREATMENT

In all the nine treatment failures due to PPNG there was little or no response to the initial drug regimen (whether with APPG or amoxycillin), and repeat treatment was also ineffective. Eighteen failures were associated with non-PPNG strains, and in six of these there was little or no response to initial treatment. In the remaining 12 cases there was an initial response (first culture results were negative), but the infection recurred within 10 days. In the 16 cases seen after the repeat treatment, failure occurred again in eight.

Retreatment with spectinomycin was successful in eight of the nine infections with PPNG that were followed and in all eight non-PPNG infections.

Discussion

It is now confirmed that a great majority of gonococcal strains in Lusaka are relatively resistant to penicillin. We believe this results from the widespread use of inadequate penicillin dosage regimens for gonorrhoea. Until recently the prevailing practice in Zambia was to treat patients with any genital ulcer or discharge with a dose of 0·6 megaunits of penicillin daily for 10 days. Antiobiotics are available free of charge and hence are widely used by medical staff for a variety of minor problems. Furthermore self-treatment with a few capsules of ampicillin is commonly practised by patients with gonorrhoea. Thus, selection of increasingly resistant gonococci is a predictable result. Despite the low susceptibility of gonococci to penicillin in vitro, however, satisfactory cure rates are still obtained with high doses of penicillin. On the other hand, the problem of PPNG appears to be more serious, since they were responsible for 33% of the treatment failures. Although their prevalence is low (3.2%) in Lusaka, all the infections were acquired within Zambia, and it is possible that some areas in the country have a higher prevalence of PPNG.

Apart from Zambia, PPNG have so far been identified in West Africa[16] and Zaire (Piot, personal communication). The size of the problem in other African countries is not yet known, but it can be presumed that the serious situation reported in a few countries is only the tip of an iceberg. Special STD clinics are established at a few teaching centres and, in general, diagnosis remains largely presumptive on clinical presentation alone and treatment practices are suboptimal. Furthermore, follow-up is poor and neither treatment failures nor sexual contacts are identified. Thus, there can be little doubt that the prevalence of PPNG will rise within a few years throughout Africa.

Treatment of PPNG infections will be a serious problem since the choice of alternative therapy is limited to a few drugs, which at the moment are prohibitively expensive for the inadequate health budgets. In addition to these problems there is little hope for an immediate remedy because many countries do not have enough trained staff and resources to carry out large-scale control programmes. The only practical approach would be to adopt a simplified control strategy within the primary health care programmes, which many countries are presently planning. STD management at all the health centres, large and small, must be improved as a priority before any community-based control is attempted. Lack of awareness and concern among clinicians is part of the problem, and medical personnel must be made aware of the special problems of gonorrhoea and be motivated to take appropriate action. Staining by Gram's method is a simple procedure that should be used routinely for initial diagnosis and for tests of cure. All treatment failures should be identified and retreated with spectinomycin.

Providers of health care in African countries must pay serious attention to the increasing problems of gonorrhoea control. Zambia has taken the first step forward in developing a small network of special clinics in the provinces, and a national STD control programme has recently been launched. As far as PPNG are concerned, it is obvious that endemic pockets in a few countries can threaten the efforts being made all over the world; hence nothing short of a concentrated global action can be expected to provide an adequate remedy.

References

1. World Health Organisation, *Technical Report Series* No 616 1978.
2. Hall WH, Schierl EA. Maccani JE. Comparative susceptibility of penicillinase-positive and -negative *Neisseria gonorrhoeae* to 30 antibiotics. *Antimicrob Agents Chemother* 1979; **15:**562-7.
3. Siegel MS, Thornsberry C, Biddle JW, O'Mara PR, Perine PL, Weisner PJ. Penicillinase-producing *Neisseria gonorrhoeae:* results of surveillance in the United States. *J Infect Dis* 1978; **137:**170-4.
4. Kibukamusoke JW, Gonorrhoea and urethral stricture. *Br J Vener Dis* 1965; **41:**135-8.
5. Verhagen AR, Gemert W. Social and epidemiological determinants of gonorrhoea in an East African country. *Br J Vener Dis* 1972; **48;**277-86.
6. Osoba AO. In: *Subfertility and Infertility in Africa*. Kwaku-Adadevoh B (ed). Ibadan: The Caxton Press Limited, 1974; 81.
7. Retel-Laurentin A. In: *Subfertility and Infertility in Africa*. Kwaku-Adadevoh B. (ed). Ibadan: The Caxton Press Limited, 1974: 69.
8. Perine PL, Biddle JW, Nsanze H, D'Costa LJ, Osoba AO, Widy-Wirsky R. Gonococcal drug resistance and treatment of gonorrhoea in Nairobi. *E Afr Med J* 1980: **57:**238-46.
9. Piot P, Van Dyck E, Colaert J, Ursi JP, Bosmans E, Meheus A. Antibiotic susceptibility of *Neisseria gonorrhoeae* strains from Europe and Africa. *Antimicrob Agents Chemother* 1979; **15:**535-9.
10. Osoba AO, Montefiore DG, Sogbetun AO, Alausa KO, Anong CN.Sensitivity pattern of *Neisseria gonorrhoeae* to penicillin and screening for β-lactamase production in Ibadan, Nigeria. *Br J Vener Dis* 1977: **53**:304-7.
11. Arya OP, Phillips I. Antibiotic sensitivity of gonococci and treatment of gonorrhoea in Uganda. *Br J Vener Dis* 1970; **46:** 149-52.
12. Plorde JJ, Kidan TG, Wright LJ. Penicillin sensitivity of gonococci in Ethiopia. *Br J Vener Dis* 1973; **49:**260-2.
13. Ratnam AV. Sexually transmitted diseases in Lusaka. *Med J Zambia* 1980; **14:**71-4.
14. Ratnam AV, Din SN, Chatterjee TK. Sexually transmitted diseases in pregnant women. *Med J Zambia* 1980; **14:**75-8.
15. Ratnam AV, Din SN, Chatterjee TK. Gonococcal infection in women with pelvic inflammatory disease in Lusaka, Zambia. *Am J Obstet Gynecol* 1980; **138:**965-8.
16. Osoba AO. Sexually transmitted diseases in tropical Africa: a review of the present situation. *Br J Vener Dis* 1981; **57:**89-94.

Br J Vener Dis 1982;**58**:32-5

Aetiology and management of non-specific vaginitis

I M DATTANI, A GERKEN, AND B A EVANS

From the Departments of Genitourinary Medicine and Medical Microbiology, Charing Cross Hospital (West London), London

SUMMARY In a study of 100 women with non-specific vaginitis, characterised by a vaginal discharge which was malodorous and pruritic in most cases and caused dyspareunia and dysuria in some, *Gardnerella vaginalis* was isolated in 46% of patients. When present, *G vaginalis* was significantly associated with *Mycoplasma hominis* and *Bacteroides* species. Isolation of *G vaginalis* was unrelated to the presenting symptoms. Treatment with povidone-iodine pessaries for two weeks produced no pronounced benefit, either clinically or microbiologically, compared with a placebo. Nevertheless, 68% of all patients followed reported improvement four weeks after the start of treatment.The findings suggest that *G vaginalis* is one cause of non-specific vaginitis, which is more likely to be seen in women using oral contraceptives and is usually cured spontaneously.

Introduction

A considerable number of young women complain of vaginal symptoms for which *Candida* species and *Trichomonas vaginalis* are not responsible. Gardner and Dukes[1] were the first to suggest that *Haemophilus vaginalis* (later renamed *Corynebacterium vaginale* and now called *Gardnerella vaginalis)* may be the cause. Since then they have been both supported[2-5] and opposed[6-9] in this view. For this study, a symptom-based approach was taken in order to establish a clinical picture for non-specific vaginitis, to determine its relationship to *G vaginalis* infection, and finally to examine the prognosis by a controlled trial with a broad-spectrum vaginal pessary, povidine-iodine, as the active treatment.

Patients and methods

STUDY POPULATION

Women complaining of vaginal discharge for at least one week were included in the study if neither candidal, trichomonal, or gonococcal infection could be detected. A total of 100 patients was seen by one of us (IMD) during 1978 at a department of genitourinary medicine.

The control group comprised 100 consecutive patients seen in the same department by the same doctor. Details of age, contraceptive method, and obstetric history were recorded. Tests for sexually transmissible disease were carried out as a routine, using standard methods for the diagnosis or exclusion of gonorrhoea, trichomoniasis, and candidosis, together with serological tests for syphilis.

CULTURE TECHNIQUES

A high vaginal swab was taken and sent to the laboratory in Stuart's transport medium. The following culture plates were inoculated: two 5% horse blood agar, one MacConkey agar, and one mycoplasma agar (base and supplement, BBL). The plates were incubated aerobically for 48 hours at 37°C, except for one blood agar plate which was placed in an anaerobic environment. The mycoplasma plates were incubated in an atmosphere of 10% CO_2 in air for five days.

IDENTIFICATION OF ORGANISMS

Organisms were identified according to standard methods.[10] *Mycoplasma hominis* was identified serologically using "taxodiscs" (BBL). *G vaginalis* was detected by the inoculation of a plate containing DST agar (Oxoid) to which 5% lysed horse blood and 2% reconstituted Isovitalex (BBL) was added. This was incubated in 10% CO_2 in air at 37°C for five days and examined daily. After 48 hours' incubation at 37°C, *G vaginalis* appeared as pinpoint grey colonies of typical short Gram-positive rods. These rapidly became Gram-variable on aging or repeated subculture. Identity was confirmed by demonstrating negative catalase, nitrate, and

Address for reprints: Dr B A Evans, Department of Genitourinary Medicine, Charing Cross Hospital (West London), Hammersmith Road, London W6 7DQ

Accepted for publication 23 July 1981

aesculin reactions. Serum peptone water sugars were inoculated and showed acid production in glucose and maltose but not in lactose, mannitol, salicin, or trehalose.

TREATMENT TRIAL

A controlled double-blind trial compared povidone-iodine pessaries (Betadine 200 mg) with a placebo of identical appearance, packaged in random-numbered containers, one pessary to be used night and morning for two weeks. Patients were asked to return four weeks after the start of treatment for both clinical and microbiological assessment. All relevant data were recorded on a study form drawn up for this purpose.

Statistical analysis was carried out by means of Yates's modification of the χ^2 test.

Results

The study group of 100 women with non-specific vaginitis had a mean age of 25·6 years (range 18-54 years); 82 were single. Forty-two women had been pregnant, 54 were using oral contraception, and 16 were fitted with an intrauterine contraceptive device (IUCD).

The control group of 100 unselected female patients had a mean age of 26·0 years (range 17-50 years); 82 were single. Forty-three had been pregnant, 39 were using oral contraception, and 18 were fitted with an IUCD. There were no ethnic differences between the two groups.

SYMPTOMS

Patients were admitted to the study group on the basis of complaint of vaginal discharge shown not to be associated with trichomoniasis, candidosis, or gonorrhoea. Additional symptoms were an offensive smell (68%) and irritation (55%); dyspareunia was noted by 35% and dysuria by 23%. The existence of a discharge was confirmed in all cases, and increased redness of the vagina was recorded in 68%. Microscopy showed polymorphonuclear leucocytes in the cervical secretions of 89%.

CULTURE

The most frequently isolated organisms in both groups of patients are shown in table I. Only *G vaginalis* and *M hominis* were isolated more frequently in the study group; this was statistically significant (χ^2_1 = 14·13 and 8·04 respectively; $P<0·001$ and $<0·01$ respectively). In addition, *Bacteroides*, an organism of equal incidence in the control group, was found in combination with *G vaginalis* in 15 patients with vaginitis compared with five controls (χ^2_1 = 4·5; P = 0·05). Therefore not only *G vaginalis* but also *M hominis* and *Bacteroides* species were significantly associated with non-specific vaginitis.

TABLE I *Organisms most frequently isolated and established pathogens. (The percentage of patients harbouring G vaginalis is shown in brackets)*

Organism	*Patients with non-specific vaginitis (n = 100)*	*Controls (n = 100)*
Gardnerella vaginalis	46	20
Mycoplasma hominis	44 (64)	24 (33)
Escherichia coli	27 (30)	29 (7)
Bacteroides species	18 (83)	18 (28)
Herpes simplex virus	4 (25)	2 (0)
Neisseria gonorrhoeae		9 (44)
Trichomonas vaginalis		14 (14)
Yeasts (mostly *Candida albicans*)		26 (15)

CORRELATION WITH SYMPTOMS AND ORAL CONTRACEPTION

The possibility that *G vaginalis* is responsible for the more severe forms of non-specific vaginitis was examined by looking for a correlation with more severe associated symptoms. From the results, it was apparent that no such relationship existed (table II).

Host factors which might relate to morbidity were also examined[9] (table III). Patients with *G vaginalis* vaginitis were much more likely to be taking oral contraceptives (χ^2_1 = 6·90; $P<0·01$) than patients harbouring *G vaginalis* in the control group. In the vaginitis group generally the relationship with oral contraception was less significant but still present (χ^2_1 = 3·94; $P<0·05$).

TABLE II *Relationship between common symptoms and isolation of G vaginalis. (Percentages are shown in brackets.)*

	Isolation of G vaginalis	
Symptoms	*Positive (n = 46)*	*Negative (n = 54)*
Offensive discharge	30 (65)	38 (70)
Pruritus vulvae	26 (56)	29 (54)
Offensive discharge or pruritus vulvae or both	39 (85)	44 (81)

TABLE III *Host factors and non-specific vaginitis. (The percentage of patients harbouring G vaginalis is shown in brackets.)*

Host factors	*Patients with non-specific vaginitis (46)*	*Controls (20)*
Oral contraception	54 (27)	39 (4)
Past pregnancy	42 (25)	43 (9)

TREATMENT TRIAL

The results of the treatment trial are summarised in table IV. In terms of clinical improvement assessed at four weeks, povidone-iodine pessaries were not significantly better than a placebo ($\chi^2_1=2\cdot38$; $P>0\cdot05$). In fact, 58% of placebo-treated patients reported improvement, and this tendency to spontaneous cure was supported by the microbiological findings; *G vaginalis* was eliminated from 62·5% of the placebo-treated group at four weeks compared with 76% of the povidone-iodine-treated group. This was not significant ($\chi^2_1 = 0\cdot29$; $P>0\cdot05$). Of the 11 patients in whom *G vaginalis* was isolated at follow up, seven (64%) reported that they were better, two patients reported no change, and two were worse, but one of these had a herpetic infection and the other a yeast infection.

TABLE IV *Effect of treatment with povidone-iodine on clinical and microbiological findings.*

Findings at 4 weeks	*No (%) of patients after treatment with:*	
	Povidone-iodine	*Placebo*
Clinical		
Improved	35 (76)	22 (58)
Not improved	11	16
Microbiological		
G vaginalis +	5	6
G vaginalis −	16 (76)	10 (62·5)

+ Present; − absent

Discussion

This study has produced a clear clinical picture of non-specific vaginitis: a whitish discharge with an offensive odour associated with pruritus vulvae, dyspareunia, and dysuria, in descending order of frequency. Moreover, a placebo-controlled trial showed that more than half the cases resolved spontaneously, both clinically and bacteriologically. Thus, it seems that specific treatment is not required in most cases of non-specific vaginitis and that if symptoms persist povidone-iodine may have some beneficial effect. Assessment at four weeks after diagnosis ensured that patients had completed a full menstrual cycle by the time they were re-examined, and thus the effects of menstruation were shared by all. The favourable outcome in the placebo-treated group suggested a possible beneficial effect from menstruation.

Nevertheless, the aetiology remains obscure. Since Gardner and Dukes first implicated *H vaginalis* in 1955, subsequent opinions have been almost equally divided on the pathogenic role of the organism. In our experience, it did not occur more frequently in the two common forms of infective vaginitis (trichomoniasis and yeast infection) than in the control group as a whole (table I), which suggests that it is not an opportunistic invader of the morbid vagina. Ingham *et al*[11] have suggested that anaerobes may enhance the pathogenicity of *G vaginalis* by interfering with phagocytosis, which gives importance to its presence in 15 out of 18 patients from whom *Bacteroides* species were isolated. *M hominis* alone may be responsible for some cases of non-specific vaginitis.[12] Pheifer *et al*[5] could find no relationship between non-specific vaginitis and *Ureaplasma urealyticum* or, for that matter, *Chlamydia trachomatis.*

Our findings in non-specific vaginitis point to *G vaginalis* as being a co-pathogen, together with *M hominis* or *Bacteroides* species or both. Exactly what circumstances favour growth of these organisms in vivo has not been established. The strong association with the presence of *Bacteroides* species, themselves no more frequent in non-specific vaginitis than in our controls, raises the possibility of anaerobic foci in the environment.

We also examined host factors and confirmed the relationship with oral contraceptives reported by McCormack *et al*[9] but not that with previous pregnancy. Oral contraception was associated not only with a greater likelihood of non-specific vaginitis but also with a much greater likelihood that the presence of *G vaginalis* would be associated with vaginal symptoms. Thus, the hormonal effects of oral contraceptives on vaginal epithelium may possibly promote the pathogenic potential of *G vaginalis* probably by enhancing its growth rate.

From our study, *G vaginalis* vaginitis seems likely to develop in a single woman who is taking an oral contraceptive, and cyclical changes in the vagina may well promote spontaneous resolution. These events may be mediated cytologically, a view supported by microscopical examination of the discharge showing cellular overgrowth by organisms, the 'clue cell' described by Gardner and Dukes.[1] An anaerobic environment may possibly be promoted by collections of cellular debris within the vagina. Although hormonal factors controlling maturation of vaginal epithelium may play a part, it seems reasonable that behavioural factors affecting vaginal hygiene could also be important in the development of non-specific vaginitis.

We would like to acknowledge Napp Laboratories Ltd for kindly supplying all the treatment used in this study, and thank the staff of the Medical Microbiology Department and the sister and staff of the Martha Clinic, West London Hospital.

References

1. Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis: a newly defined specific infection previously classified "non-specific" vaginitis. *Am J Obstet Gynecol* 1955;**69**:962-76.
2. Brewer JI, Halpern B, Thomas G. *Haemophilus vaginalis* vaginitis. *Am J Obstet Gynecol* 1957;**74**:834-8.
3. Dunkelburg WE jun, Hefner JD, Patow WE, Wyman FJ jun, Orup HI. *Haemophilus vaginalis* among asymptomatic women. *Obstet Gynecol* 1962;**20**:629-36.
4. Josey WE, McKenzie WJ, Lambe DW jun. *Corynebacterium vaginale (Haemophilus vaginalis)* in women with leukorrhea. *Am J Obstet Gynecol* 1976;**126**:574-8.
5. Pheifer TD, Forsyth PS, Durfee MA, Pollock HM, Holmes KK. Non-specific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. *N Engl J Med* 1978; **298**:1429-33.
6. Heltai A, Taleghany P. Non-specific vaginal infections. A critical evaluation of *Haemophilus vaginalis*. *Am J Obstet Gynecol* 1959;**77**:144-51.
7. Frampton J, Lee Y. Is *Haemophilus vaginalis* a pathogen in the female genital tract? *Journal of Obstetrics and Gynaecology of the British Commonwealth*, 1964;**71**:436-44.
8. Corbishley CM. Microbial flora of the vagina and cervix. *J Clin Pathol* 1977;**30**:745-8.
9. McCormack WM, Hayes CH, Rosner B, *et al*. Vaginal colonisation with *Corynebacterium vaginale (Haemophilus vaginalis)*. *J Infect Dis* 1977;**136**:740-5.
10. Cowan ST, Steel KJ. *Manual for the identification of Medical Bacteria*. 2nd ed. London: Cambridge University Press, 1974.
11. Ingham HR, Sisson PR, Tharagonnet D, *et al*. Inhibition of phagocytosis in vitro by obligate anaerobes. *Lancet* 1977; ii:1252-4.
12. McCormack WM, Braun P, Yhu-Hsiung L, Klein JO, Kass EH. Medical progress: the genital mycoplasmas. *N Engl J Med* 1973;**2**:78-88.

Br J Vener Dis 1982;**58**:36-9

Quantitative study of Chlamydia trachomatis in genital infection

H MALLINSON,* O P ARYA,† AND A D GODDARD‡

*From the *Regional Public Health Laboratory, the †University Department of Venereology, Royal Liverpool Hospital, and the ‡Computer Laboratory, University of Liverpool, Liverpool*

SUMMARY *Chlamydia trachomatis* inclusion counts on inoculated McCoy cell coverslips were used as an index of the degree of infection of the cervix in women and of the urethra in men with urethritis. High inclusion counts were obtained significantly more often from men than from women, from women with cervical ectopy, and from women who had had recent sexual intercourse. Low inclusion counts were significantly more common in men with a past history of gonococcal urethritis.

Higher chlamydial isolation rates in women with gonorrhoea and in women taking the contraceptive pill could not be attributed to a greater degree of infection, since inclusion counts were not raised in these patients. There was evidence that strains of *C trachomatis* might vary in their ability to establish themselves in the genital tract because high counts in men with NGU were associated with high counts in their female consorts and the levels of counts in men were associated with the frequency of chlamydial isolation from their female consorts.

The relatively simple technique of inclusion counts in cultures for chlamydia from the genital tract may yield valuable information about the behaviour of different strains of *C trachomatis* in causing pathological changes, in the transmission of infection between individuals, and in the response to specific chemotherapy.

Introduction

When *Chlamydia trachomatis* is grown in McCoy cell tissue culture the number of intracellular inclusion bodies that develop after 2-3 days directly reflects the number of viable particles in the original inoculum. Inclusion counts in cultures from the genital tract may thus be used to assess the degree of infection.[1] In a survey of patients attending a sexually transmitted diseases (STD) clinic we have graded chlamydia-positive cultures into those with low, high, or very high inclusion counts and related these to epidemiological and clinical aspects of the infections.

Patients and methods

STUDY POPULATION

This comprised all men with urethritis and all women who attended the STD clinic at St James's Hospital, Birkenhead, on Monday afternoons or Thursday mornings from January 1976 to September 1978. Patients who had received an antibiotic or sulphonamide in the past month and men who had reported urethritis within the past three months were excluded.

CLINICAL AND CULTURAL TECHNIQUES

Demographic data, past history, history of recent exposure, symptoms, and signs were recorded on precoded proformas. Details of the examination of female patients and cultural methods, including the isolation of *C trachomatis* on idoxuridine-treated McCoy cell coverslips, have been described.[2 3] Urethral swabs from men were cultured for *Neisseria gonorrhoeae* and *C trachomatis* and used to prepare smears for identifying pus cells and Gram-negative intracellular diplococci. Urethritis was diagnosed when smears showed $\geqslant 15$ polymorphonuclear leucocytes (PMNL) in each of five microscope fields (× 100 objective, × 6·3 eye-pieces).

CHLAMYDIAL INCLUSION COUNTS

Chlamydia-positive coverslips (12mm in diameter) were graded as showing low or high inclusion counts

Address for reprints: Dr H Mallinson, Regional Public Health Laboratory, Fazakerley Hospital, Lower Lane, Liverpool L9 7AL

Accepted for publication 23 August 1981

by scanning across several diameters with the × 20 objective and × 10 eye-pieces of the dark-ground microscope. Under these conditions each traverse of a coverslip diameter covered one twenty-fourth part of the whole coverslip area.

Low counts: Less than 5 inclusions per diameter was equivalent to <120 inclusions per whole coverslip; such coverslips were graded as giving low inclusion counts.

High counts: Coverslips with ≥5 inclusions per diameter (≥120 inclusions per coverslip) were graded as giving high inclusion counts.

Very high counts: Some coverslips had many inclusions in every microscope field (one nine-hundredth of the coverslip area); those with ≥5 inclusions per field were graded as giving very high (≥4500) inclusion counts.

STATISTICAL ANALYSIS

The data were analysed with a computer using the 'statistical package for social sciences'. Tests of significance were performed by the χ^2 method applying Yates's correction where appropriate.[4]

Results

C trachomatis was isolated from 147 of 474 (31%) women and from 191 of 413 (46%) men with urethritis. Inclusion counts were performed on a random selection of 115 women and 181 men (tables I and II). Overall, high counts (≥120) were found in about half (156 or 53%) and very high counts (≥4500) in almost one-fifth (52 or 18%) of these 296 positive coverslips. Swabs with high counts were obtained significantly more often from the chlamydia-positive men with urethritis (57%) than from the chlamydia-positive women (45%) ($\chi_1^2 = 4 \cdot 23$, $P < 0 \cdot 05$).

WOMEN

The distribution of inclusion counts was not affected by age, marital state, or stage of menstrual cycle.

Although isolation rates for chlamydia were significantly higher ($\chi_1^2 = 13 \cdot 3$, $P < 0 \cdot 001$) in those using oral contraceptives (40% chlamydia-positive) than in those not practising contraception (21% chlamydia-positive) the use of oral contraceptives did not influence the incidence of high inclusion counts. An

TABLE I *Factors studied in relation to C trachomatis inclusion counts in women*

		No (%) of patients with following inclusion counts:		
Factor examined	*Total No of patients*	*Low (<120)*	*High (≥120)*	*Very high (≥4500)*
All women	115	63 (55)	52 (45)	17 (15)
Age (years)				
15-19	33	19 (58)	14 (42)	6 (18)
20-29	68	36 (53)	32 (47)	9 (13)
≥30	14	8 (57)	6 (43)	2 (14)
Contraceptive method				
Pill	82	44 (54)	38 (46)	11 (13)
None	21	13 (62)	8 (38)	3 (14)
Last intercourse				
<1 week	37	12 (32)	25 (68)	8 (22)
1-2 weeks	53	29 (55)	24 (45)	8 (15)
≥3 weeks	25	22 (88)	3 (12)	1 (4)
Cervical ectopy				
Absent	29	21 (72)	8 (28)	2 (7)
Simple	43	24 (56)	19 (44)	5 (12)
Hypertrophic	43	18 (42)	25 (58)	9 (21)
T vaginalis				
Present	18	14 (78)	4 (22)	2 (11)
Absent	97	49 (51)	48 (49)	15 (16)
Candidosis				
Present	19	11 (58)	8 (42)	3 (16)
Absent	96	52 (54)	44 (46)	14 (15)
Gonorrhoea				
Present	47	25 (53)	22 (47)	6 (13)
Absent	68	38 (56)	30 (44)	11 (16)
Cervical mucopus:				
With gonorrhoea				
Present	13	4 (31)	9 (69)	2 (15)
Absent	34	21 (62)	13 (38)	4 (12)
Without gonorrhoea				
Present	17	9 (53)	8 (47)	3 (18)
Absent	51	29 (57)	22 (43)	8 (16)
Residual symptoms after treatment for gonorrhoea				
Present	12	5 (42)	7 (58)	3 (25)
Absent	32	18 (56)	14 (44)	4 (13)

TABLE II *Factors studied in relation to C trachomatis inclusion counts in men with urethritis*

		No (%) of patients with following inclusion counts:					
Factor examined	*Total No of patients*	*Low (<120)*		*High (≥120)*		*Very high (≥4500)*	
All men with urethritis	181	77	(43)	104	(57)	35	(19)
Age (years)							
15-19	22	5	(23)	17	(77)	7	(32)
20-29	108	51	(47)	57	(53)	19	(18)
≥30	51	21	(41)	30	(59)	9	(18)
Men with							
GU	45	22	(49)	23	(51)	6	(13)
NGU	136	55	(40)	81	(60)	29	(21)
Past history of NGU*							
Present	53	24	(45)	29	(55)	10	(19)
Absent	127	53	(42)	74	(58)	25	(20)
Past history of GU*							
Present	43	24	(56)	19	(44)	3	(7)
Absent	137	53	(39)	84	(61)	32	(23)
Men with NGU whose consorts were:							
Chlamydia −	14	9	(64)	5	(36)	1	(7)
Chlamydia + (<120)	9	4	(44)	5	(56)	2	(22)
Chlamydia + (120-4500)	8	3	(38)	5	(62)	3	(38)
Chlamydia + (>4500)	5	1	(20)	4	(80)	2	(40)

+ Positive; − negative; NGU = non-gonococcal urethritis; GU = gonococcal urethritis
*Information not recorded for one patient

effect of recent intercourse was seen in that high counts ($\chi^2_1 = 11 \cdot 0$, P<0·001) or very high counts ($\chi^2_1 = 4 \cdot 21$, P<0·05) were obtained significantly more often compared with low counts in those who reported intercourse within the previous week.

High counts were significantly associated with the presence of ectopy ('erosion') ($\chi^2_1 = 3 \cdot 96$, P<0·05), with very high counts being found more frequently in patients whose ectopy was hypertrophic.

Low counts were more common in those with concurrent *Trichomonas vaginalis* infection than in those without ($\chi^2_1 = 3 \cdot 51$, P>0·05) but counts were not affected by concurrent *Candida* infection. Although isolation rates for chlamydia were significantly higher ($\chi^2_1 = 16 \cdot 2$, P<0·001) in those with gonorrhoea (44% chlamydia-positive) than in those without (25% chlamydia-positive), the distribution of inclusion counts was unaffected by concurrent gonococcal infection. In women with gonorrhoea, however, cervical mucopus was associated with high counts, but these were not found in the presence of mucopus without gonococcal infection. Women who complained of persistent residual symptoms after treatment for gonorrhoea included more with high counts initially than those in whom symptoms resolved quickly.

MEN

High counts were more common in men under 20 years than in those over, but the difference was not significant ($\chi^2_1 = 3 \cdot 16$, P>0·05). In men with non-gonococcal urethritis (NGU) high and very high counts were more frequent than in those with gonococcal urethritis (GU) but the difference was not significant. In both groups a past history of NGU did not affect the distribution of counts but a history of GU was associated with a reduction in the frequency of high counts ($\chi^2_1 = 3 \cdot 91$, P<0·05) or very high counts ($\chi^2_1 = 4 \cdot 61$, P<0·05).

In men with NGU, high counts were associated with high counts in their female consorts. There was also a link between counts in men and the frequency of chlamydial isolation from their female consorts. Of eight men with very high counts, seven (88%) had consorts infected with *C trachomatis;* of 11 men with high but not very high counts, seven (64%) had infected consorts; of 17 men with low counts, eight (47%) had infected consorts.

Discussion

Chlamydia trachomatis is an organism that is commonly transmitted by sexual intercourse and from mothers to new-born infants. It causes urethritis in men, conjunctivitis and pneumonitis in babies, and infection of the genital tract in women which is often symptomless and not associated with any detectable pathological lesion. It is clearly important to establish whether the numbers of chlamydia present in various infected or carrier sites have a bearing on the pathology and epidemiology of *C trachomatis* infection.

Hilton *et al*[5] found that women taking oral contraceptives had a significantly higher proportion of specimens with more than 100 inclusions per coverslip than did women not taking the contraceptive pill. In a study of women consorts of men with NGU, Tait *et al*[6] found higher mean inclusion counts to be associated with the use of oral contraceptives or the presence of endocervical mucopus but not with hypertrophic ectopy. In a much larger study, however, Hobson *et al*[1] confirmed the association of high counts (>1000) with cervical mucopus but found in contrast that high counts were associated with the presence of cervical ectopy but not directly with the use of the contraceptive pill. Our present results support this latter finding of high counts in those with cervical ectopy and a lack of effect on the count of the contraceptive pill. The tendency to find high counts in those with cervical mucopus was apparent only in women with gonorrhoea. A reported association of high counts with younger age in women[1] was not confirmed in this study, although a similar trend was suggested in men with urethritis. The significantly higher chlamydial isolation rates in women with gonorrhoea and those taking the contraceptive pill could not be attributed to a greater degree of infection since inclusion counts were not found to be raised in these patients.

In men it is not clear why fewer high counts were obtained from patients with a history of gonococcal but not non-gonococcal urethritis. There was an association between high counts in men and in their female consorts and between inclusion counts in men and the frequency with which chlamydia were isolated from their female consorts. This suggests that some strains of *C trachomatis* may establish themselves in the genital tract more readily than others and this may be a factor determining the numbers of organisms present.

The estimation of inclusion counts would be unlikely to improve the routine management of individual patients with STD. A knowledge of both isolation rates and the distribution of inclusion counts in a patient population should, however, allow a clearer understanding of *C trachomatis* infections. It may yield valuable information on the effect of strain variation in causing pathological changes in the genital or respiratory tracts, on the transmission of infection from one individual to another, on the response to specific chemotherapy, and on the relation to other features in the genital tract, such as associated infections including gonorrhoea, trichomoniasis, and candidosis.

We are indebted to Dr G C Turner for help and advice in preparing the manuscript.

References

1. Hobson D, Karayiannis P, Byng RE, Rees E, Tait IA, Davies J. Quantitative aspects of chlamydial infection of the cervix. *Br J Vener Dis* 1980;**56**:156-62.
2. Arya OP, Mallinson H, Goddard AD. Epidemiological and clinical correlates of chlamydial infection of the cervix. *Br J Vener Dis* 1981;**57**:118-24.
3. Mallinson H, Sikotra S, Arya OP. A cultural method for large-scale screening for *Chlamydia trachomatis* genital infection. *J Clin Pathol* 1981;**34**:712-8.
4. Swinscow TDV. Statistics at square one: XV The χ^2 tests (continued). *Br Med J* 1976;ii:513-4.
5. Hilton AL, Richmond SJ, Milne JD, Hindley F, Clarke SKR. *Chlamydia* A in the female genital tract. *Br J Vener Dis* 1974;**50**:1-10.
6. Tait IA, Rees E, Hobson D, Byng RE, Tweedie MCK. Chlamydial infection of the cervix in contacts of men with non-gonococcal urethritis. *Br J Vener Dis* 1980;**56**:37-45.

Br J Vener Dis 1982;**58**:40-3

Does psychiatric illness affect the recurrence rate of genital herpes?

DAVID GOLDMEIER AND ANTHONY JOHNSON

From the Department of Genitourinary Medicine, University College Hospital, London; and the MRC Biostatistics Unit, Medical Research Council Centre, Cambridge

SUMMARY The progress of 58 patients with first attacks of genital herpes was monitored for up to 30 weeks. The effect of sex (gender), age, social class, and non-psychotic psychiatric illness (as measured by the "general health questionnaire," GHQ) on the recurrence rate of herpes was studied. The recurrence rates, measured by the actuarial percentage recurrence-free curves, showed that the 29 patients with GHQ scores above 11 had a significantly higher recurrence rate than those who scored less than 11 (P=0·002). There were no important differences between recurrence rates according to sex, age, or social class.

These findings suggest that non-psychotic states, such as anxiety or obsessionality resulting in GHQ scores above 12, may possibly cause an excess production of adrenergic substances which encourages reactivation of latent genital herpes. Thus, to lessen the recurrence rate in such patients, a psychological or chemical approach to treatment could be used to modify the autonomic sympathetic response.

Introduction

Recurrences of genital herpes are painful and worrying and may be precipitated by various factors, including sexual intercourse, menstruation, and the emotional state of the patient.[1] Clinical episodes of recurrent herpes seem to be more common in male than in female patients,[2] and a recurrence is more likely if the primary attack was due to type 2 rather than type 1 virus.[3] Our experience suggests that patients who experience minor depression, anxiety states, feelings of inadequacy, and hypochondriasis have a first recurrence sooner than euthymic patients.

The "general health questionnaire" (GHQ)[4] measures non-psychotic psychiatric illness over the few weeks before interview, a high score indicating a high possibility of such illness. The GHQ is not a personality test; it has been validated both in general practice[5] and in the medical outpatient department of a general hospital.[4] GHQ scores have been shown to correlate well (+ 0·8) with psychiatrists' independent assessments and correctly identify 95% of patients thought to be psychiatrically ill at interview. The questionnaire is self-administered and consists of 60 questions about feelings and behaviour. The patient scores one for each symptom experienced "over the past four weeks," so that the total score may range from 0 to 60. The questionnaire takes approximately 15 minutes to complete. Patients with scores in excess of 11 are defined as "potential psychiatric cases" (although they may have overt illness). Using the GHQ as a quantitative assessment of the mental state of the patient, we tested the hypothesis that the rate of recurrence of genital herpes after the primary attack was higher in patients with pre-existing psychiatric illness than in those without.

Patients and methods

STUDY POPULATION

One hundred and thirty male and female patients seen at the department of genitourinary medicine, University College Hospital, London, between June 1979 and June 1980 who had their first clinical attack of genital herpes confirmed by culture were considered for entry into the study. They were excluded if any of the following criteria were satisfied: they could not reattend or keep in touch by post (four patients); they had a past history of genital herpes (eight patients); they spoke insufficient English to answer a questionnaire (two patients);

Address for reprints: Dr D Goldmeier, Department of Genitourinary Medicine, University College Hospital, London WC1E 6AU

Accepted for publication 25 August 1981

they were married (in a small-pilot survey married patients were unwilling to have postal inquiries sent to them) (10 patients); they had painless genital ulcerations (one patient with early syphilis); they were homosexual men with painful anorectal ulceration (12 patients); they had ulceration and had returned from Africa or the Far East in the preceding three months (two patients); or they had concomitant genital disease, for example gonorrhoea, chlamydial infection, trichomoniasis, candidosis, or warts (30 patients). Three patients refused to take part in the study.

The nature of the study was explained to the potential participants and their informed consent obtained to complete the GHQ and for follow-up inquiry by letter. Age, occupation, and sex of the 58 patients who were eligible to take part in the study were recorded. Those who agreed to take part were told to bathe the lesions frequently with saline. No antiviral agents were used.

INITIAL EXAMINATION

Serum from the genital ulcers was transferred on alginate swabs (Medical Wire and Equipment Co Ltd, ref: MW-104) into virus transport medium,[6] refrigerated at 4°C for not more than four hours, and transferred on to a cell culture system[7] to isolate herpes simplex virus. The isolates were not typed. Serum from the ulcers was also examined by dark-field microscopy for *Treponema pallidum*. Serological tests (rapid plasma reagin and *T pallidum* haemagglutination assay[8]) were routinely performed to exclude syphilis.

The 60-item GHQ[4] was explained to the patients, who were asked to complete it alone in a side-room. Patients were told that if they failed to reattend for follow up, their progress would be monitored by a postal questionnaire.

FOLLOW-UP INVESTIGATIONS

Dark-field microscopy was repeated twice in the following seven days and again at two and four weeks after the initial examination if the ulcers had not healed. Serological tests were repeated at four weeks and again at three months after the initial visit for patients who reattended. Patients who returned to the clinic because of painful genital ulceration had further cultures performed for herpes simplex virus, repeat dark-ground microscopy to exclude *Treponema pallidum,* and repeat serology for syphilis.

Postal inquiries were sent out at 14 and 28 weeks after the inital visit to patients who had not reattended. These asked about recurrence (including date) of genital sores during the period from the first visit. Reminder letters were sent out at 16 and 30 weeks to the patients who did not respond to the postal inquiries. Personal letters were sent to the 13 patients who still failed to reply.

STATISTICAL ANALYSIS

For each patient the period from the first clinic visit to either recurrence or the date last known to be recurrence-free was calculated to the nearest week. The date of recurrence was defined as the date of virologically confirmed recurrence in those patients who reattended the clinic and the date of first appearance of symptoms of recurrence (as recorded by the patient on the postal questionnaire) in those patients who did not reattend the clinic.

The actuarial percentages recurrence-free[9] at weekly intervals after the initial clinic visit were calculated for various subdivisions of the sample. The resulting percentage recurrence-free curves were compared using the logrank test.[9] The distributions of GHQ scores in the subgroups of patients with recurrence confirmed by culture, recurrence not confirmed by culture, and no recurrence at 28 weeks were compared using the Kruskal-Wallis[10] one-way analysis of variance by ranks. Comparisons of these distributions in any two subgroups were made using the Mann-Whitney U statistic.[10]

Results

Thirty-seven women and 21 men with primary genital herpes took part in the study and 29 (50%) reported a recurrence within 28 weeks of attendance. Seven of these 29 (24%) patients returned to the clinic during the 28 weeks because they had painful genital ulceration; all were culture-positive. Recurrence was not confirmed by culture in the remaining 22 patients who did not reattend the clinic.

Of the 29 patients who did not report recurrence of genital herpes, 16 (55%) stated in response to a postal questionnaire that they were recurrence-free 28 weeks after the initial visit, five (17%) were recurrence-free at 14 weeks but did not respond to the questionnaire at 28 weeks, and the remaining eight (28%) patients were lost to follow-up after their initial attendance for the primary attack. These eight patients all attended for at least the first week after the initial visit but did not reply to the postal inquiries at 14, 16, 28, and 30 weeks or to a personal letter.

For the total of 58 patients there were no important differences between the actuarial percentage recurrence-free curves for the two sexes (logrank test $\chi_1^2 = 0\cdot39$, $P = 0\cdot53$), for the three age groups (<24, 25-34, 35 years, and over) ($\chi_2^2 = 2\cdot1$, $P = 0\cdot34$), or the five social classes ($\chi_4^2 = 1\cdot1$, $P = 0\cdot89$). The 29 patients with a GHQ score above 11 had a significantly higher recurrence rate than the

29 patients with a GHQ of 11 or less (logrank test $\chi^2_1 = 9\cdot 9$, $P = 0\cdot 002$). The actuarial percentage recurrence-free curves for these two groups are shown in the figure.

The characteristics of three subgroups of patients (recurrence confirmed by culture, recurrence unconfirmed by culture, and recurrence-free at 28 weeks from entry) are summarised in the table. The distributions of GHQ scores in these three groups were significantly different (Kruskal-Wallis test $\chi^2_2 = 10\cdot 8$, $P = <0\cdot 01$). Subsequent analysis by the Mann-Whitney test indicated that the group without recurrence at 28 weeks had lower GHQ scores than the other groups ($P<0\cdot 01$ in both comparisons); there was no important difference between the distributions of GHQ scores in the two recurrence groups ($U = 76\cdot 5$, $P = 0\cdot 98$).

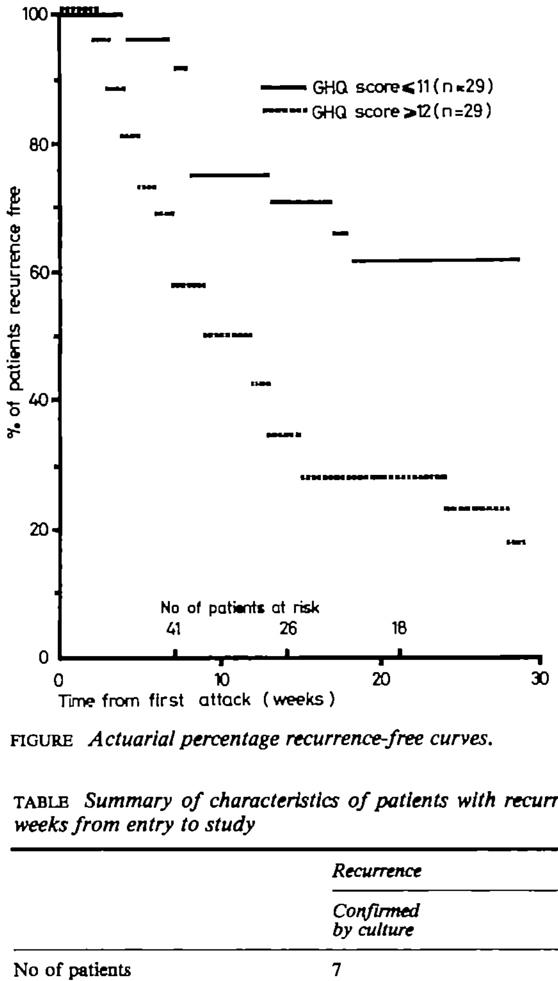


FIGURE *Actuarial percentage recurrence-free curves.*

Discussion

Although the isolates were not typed it seems from the present study there is no association between age, sex, social class, and time to recurrence of genital herpes. The recurrence rate in potential psychiatric cases (those scoring 12 or over on the GHQ), however, was significantly greater than the 'normals' (11 or less). Similarly, the GHQ scores of patients with recurrences were significantly greater than those who felt they did not have a recurrence during the 28 weeks of the study.

Because the GHQ is a measure of hypochondriasis as well as depression and anxiety, it may be argued that the group with recurrence unconfirmed by culture would be likely to feel they had a recurrence when in fact they did not. There was no important difference, however, between the distribution of the GHQ scores in the subgroups with recurrence confirmed by culture and recurrence unconfirmed by culture. We consider this, together with the identical subjective feeling of disease relapse, to be evidence in favour of these two groups being similar psychologically and in terms of mode of clinical

TABLE *Summary of characteristics of patients with recurrence of genital herpes and patients with no recurrence at 28 weeks from entry to study*

	Recurrence		
	Confirmed by culture	*Not confirmed by culture*	*No recurrence at 28 weeks**
No of patients	7	22	16
Sex			
Male	3	7	7
Female	4	15	9
Social class			
I	1	5	3
II	2	9	4
III	2	6	7
IV	2	1	1
V		1	1
Age (years)			
Range	21-38	18-38	19-41
Median	23	24·5	23
GHQ score			
Range	7-50	0-46	0-26
Median	18	22·5	5

*The 5 patients with no recurrence by 14 weeks and the 8 lost to follow-up are excluded from this table.

recurrence. Thus, most of the patients with recurrence unconfirmed by culture would have been culture-positive if they had revisited the clinic at the time of apparent recurrence.

The results of this study suggest that ongoing potential non-psychotic psychiatric illness contributes towards reactivation of latent genital herpes. This of course assumes that the GHQ score in any one patient remains essentially static up to the time of recurrence. Juel-Jenson[11] has stated that 'emotional upset' may be a factor causing reactivation of latent infection 'possibly due to release of adrenaline.' Recurrences of herpes simplex encephalitis and keratitis are known to be precipitated in rabbits by the administration of parenteral adrenaline.[12 13] In many of the states, such as anxiety or obsessionality, which result in a patient having a GHQ score over 12, an excess production of adrenergic substances may possibly encourage clinical relapse of genital herpes. Modification of autonomic sympathetic response, whether by psychological or chemical means, may thus lessen the recurrence rate in patients who experience recurrences of genital herpes and who have minor non-psychotic mental disability.

We should like to thank Dr J D Oriel for allowing us access to his patients and for his time and effort with advice at all stages of this work. Our thanks are also due to the Microbiology Department at University College Hospital for their services.

References

1. Hutfield DC. Genital herpes. *Br J Vener Dis* 1968;**44**:241-50.
2. Corey L. Clinical epidemiology and clinical course of genital herpes simplex virus infection. In: *Non-gonococcal Urethritis and other Sexually Transmitted Diseases of Public Health Importance.* Report of WHO Scientific Group, WHO Technical Report Series No 6600. Geneva: WHO, 1981:21-30.
3. Corey L, Stamm W, Reeves WC. Controlled trial of BCG vaccination for the prevention of recurrent genital herpes. *Antimicrobial Agents and Chemotherapy, 1976.* Bethesda, Maryland: American Society for Microbiology, 1979.
4. Goldberg DP. *The Detection of Psychiatric Illness by Questionnaire.* Oxford: Oxford University Press, 1972.
5. Goldberg DP, Blackwell B. Psychiatric illness in a suburban general practice. A detailed study using a new method of case identification. *Br Med J* 1970;**2**:439-43
6. Stokes EJ, Ridgway GL. In: *Clinical Bacteriology,* 5th ed. London: Arnold, 1980; 365.
7. Herrman EC, jun. Experiences in laboratory diagnosis of herpes simplex, varicella zoster and vaccinia virus infections in routine medical practice. *Mayo Clin Proc* 1967;**42**:744-53.
8. Stokes EJ, Ridgway GL. In: *Clinical Bacteriology.* 5th ed. London: Arnold, 1980; 274-83.
9. Peto R, Pike MC, Armitage P, *et al.* Design and analysis of randomised clinical trials requiring prolonged observation of each patient. II Analysis and examples. *Br J Cancer* 1977;**35**:1-39.
10. Siegal S. *Non-parametric Statistics for the Behaviour Sciences.* New York: McGraw-Hill, 1956.
11. Juel-Jenson BE. A new look at infectious diseases. Herpes simplex and zoster. *Br Med J* 1973;**i**:406-10.
12. Tokumaru T, Scott TFM. The herpes virus group; herpes virus hominis, herpes virus simiae, and herpes virus suis. In: Lennette EH, Schmidt NJ, eds. *Diagnostic Procedures for Viral and Rickettsial Diseases.* 3rd ed. New York: American Public Health Association Inc, 1964; 381-433.
13. Laibson PR, Kibrick S. Reactivation of herpetic keratitis by epinephrine in rabbits. *Arch Ophthalmol* 1966;**75**:254-60.

Br J Vener Dis 1982;**58**:44-7

Incidence of herpes simplex virus types 1 and 2 isolated in patients with herpes genitalis in Sheffield

I G BARTON,* G R KINGHORN,† S NAJEM,* L S AL-OMAR,* AND C W POTTER*

*From the *Department of Virology, University of Sheffield Medical School; and the †Department of Genitourinary Medicine, Royal Infirmary, Sheffield*

SUMMARY Thirty-one strains of herpesvirus (HSV), isolated from patients presenting with the clinical features of herpes genitalis, were typed by polypeptide analysis of virus proteins in sodium dodecyl sulphate polyacrylamide gels. Nineteen (61·3%) of the isolates were shown to be HSV type 1 and 12 (38·7%) HSV type 2. There was no obvious difference in the incidence of HSV-1 in primary or recurrent infections and no apparent correlation between the genital site of isolation and virus type. The high incidence of genital HSV-1 infection in this group of patients is probably due to the increased practice of oro-genital contact and has possible implications for the future development of drugs and vaccines in the control of genital herpes.

Introduction

Herpesvirus hominis (HSV) is becoming an increasingly common cause of genital infections. Despite the development of circulating antibodies to the virus many people develop recurrent infections, usually at or close to the site of primary infection but sometimes remote from it.[1]

Herpesvirus hominis may be divided into HSV type 1 (HSV-1) and HSV type 2 (HSV-2) by serological criteria, and it is generally accepted that there is a difference in the site of involvement and modes of transmission for the two serotypes.[2 3] Thus, HSV-1 is usually transmitted non-venereally and affects non-genital sites, including the mouth, lips, skin above the waist, eyes, and brain; HSV-2 is most often transmitted venereally causing infection of the genitalia and skin below the waist. Nahmias and Starr[1] found that 89% of female and 97% of male urogenital infections were due to type-2 virus. Other reports, however, indicate that HSV-1 may play a greater part in genital infections than was previously thought. In a survey in Edinburgh, Smith, Peutherer, and Robertson[4] found 14 of 64 (22%) female and 11 of 124 (9%) male genital isolates to be HSV-1; in the United States Kaufman[5] isolated HSV-1 from nine of 67 (13·4%) genital infections; and in a Japanese study 23 of 53 (43%) genital isolates from female patients were found to be HSV-1.[6]

Previous studies of the incidence of HSV-1 in genital infections have relied on the use of kinetic or quantal neutralisation tests or immunofluorescence to type the virus[7 8]; these tests are often difficult to perform, and the results are not always clear-cut. In this study viruses were typed by analysis of the polypeptides present in infected cells using sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE), since there are many reports of distinct intertypic differences between HSV-1 and HSV-2 structural and infected cell polypeptides.[9 10] This technique allows unequivocal identification of HSV-1 and HSV-2 strains. In addition, 25 of the isolates described in this report have also been typed by digestion of virus DNA with restriction enzymes and separation of the resultant fragments on agarose gels. Since HSV-1 and HSV-2 virus DNA have been shown to have no coincident sites for any of the restriction enzymes which have been tested, they give easily identifiable patterns on electrophoresis, and this typing method again gives unequivocal results.[11-13] Using these techniques the incidence of HSV-1 and HSV-2 in genital lesions was studied in a series of patients attending the sexually transmitted diseases clinic in Sheffield.

Materials and methods

TISSUE CULTURE

BHK-21 (C13) cells (obtained from Flow Laboratories, Irvine, Scotland) were grown as monolayers in Glasgow modified Eagle's medium containing 10% (w/v) tryptose phosphate broth and 10% (v/v) newborn-calf serum. Cells were subcultured at regular intervals on a split ratio basis.

Address for reprints: Dr I G Barton, Department of Virology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX

Accepted for publication 6 July 1981

VIRUSES

HSV-1 (71-15) and HSV-2 (333) (obtained from F Rapp, Milton S Hershey Centre, Pennsylvania, USA) were propagated at low multiplicity in BHK-21 cells.

ISOLATION FROM SWABS

Swabs were taken of vesicular fluid or secretions from ulcerated genital lesions and immediately placed in a transport medium. On receipt this was divided into aliquots and stored at −80°C until used for inoculation of cells.

Confluent cultures of cells in 2-oz or 4-oz glass medical flats were inoculated with 0·5-1·0 ml of transport medium. The inoculum was adsorbed for one hour at 37°C, the monolayer washed once with Eagle's minimal essential medium containing 2% (v/v) newborn-calf serum (EMM), and 10 ml EMM then added to each bottle. The cells were maintained at 37°C with changes of medium every 3-5 days and observed daily for cytopathic changes (CPE). The monolayers were observed for a minimum of 14 days or until cell degeneration occurred. Cultures which showed 75% CPE were frozen at −80°C until harvested.

PREPARATION OF VIRUS STOCKS

Positive cultures were thawed at 37°C, the cells scraped into the medium, and the cell suspension sonicated at full power for 30 seconds using an MSE ultrasonic disintegrator. After cell disruption to release intracellular virus, the suspension was centrifuged for 10 minutes at 2000 × *g* and the supernatant containing the released virus stored in 1-2 ml aliquots at −80°C. Viruses were passed 1-3 times in BHK-21 to increase the titre; the final virus pool was prepared by inoculating confluent monolayers of BHK-21 cells in 4-oz medical flats with 0·01-0·1 plaque-forming units (pfu) per cell, and the cells harvested after 48 hours' incubation.

VIRUS TITRATION

For the determination of an infectivity titre, confluent monolayers in 5-cm plastic Petri dishes (Sterilin, Middlesex) were inoculated with 0·1 ml of an appropriate virus dilution. The virus was allowed to adsorb for one hour at 37°C and then 5 ml of EMM containing 0·8% carboxymethylcellulose was added to each dish. The dishes were further incubated at 37°C in an atmosphere of 5% CO_2 in air for 3-4 days; after this time the cells were fixed with 10% formol saline, stained with Leishman's stain, and the plaques counted.

LABELLING OF INFECTED CELL POLYPEPTIDES

The labelling and preparation of infected cell polypeptides with ^{35}S-methionine in BHK-21 cells was performed as described by Lonsdale *et al.*[14]

GEL ELECTROPHORESIS

Samples containing equal numbers of trichloracetic acid precipitable counts were analysed on 18 × 14 cm slab gels of 8·5% acrylamide with a 5% stacking gel using the discontinuous buffer system of Laemelli.[15] A set of proteins of known molecular weight (obtained from Pharmacia, Hounslow, Middlesex) were included in each gel together with the ^{35}S-methionine-labelled infected cell polypeptides of the laboratory strains of HSV-1 (71-15) and HSV-2 (333). Electrophoresis of the gels and the procedures used in staining and destaining were the same as described by Lonsdale *et al.*[14] Gels were dried under vacuum using a Bio-Rad gel drier and exposed to *x*-ray film for varying lengths of time.

PATIENTS

The first 21 specimens were taken from consecutive patients with clinically obvious genital herpes seen between March and May 1980. This selection favoured the inclusion of patients with severe primary genital infections. The remaining 10 patients were seen between 29 October and 11 November 1980; during this period consecutive patients who presented with any form of genital ulceration were routinely tested for herpes virus. Except for patients 13 and 28 the cases were unrelated.

Results

VIRUS IDENTIFICATION

Virus samples labelled with ^{35}S-methionine were electrophoresed in 8·5% polyacrylamide gels as described above; a typical gel is shown in the figure. The principal feature used to identify the strain of virus was the molecular weight of the major capsid protein which is greater in HSV-2 than HSV-1.[9 10] All the strains of HSV-1 analysed also gave rise to a heavily labelled band of molecular weight 122K which was absent from all the strains of HSV-2 examined. Although several differences between both virion and infected cell polypeptides have been reported,[9 10] these were not always apparent in the gel system used. To confirm the accuracy of this method of typing the virus, 25 isolates were also typed by the restriction enzyme technique of Lonsdale.[16] In all the 25 isolates examined the polypeptide and restriction endonuclease analysis were in agreement.

CLINICAL ISOLATES

A total of 31 isolates from both male and female patients was typed; the source and the identification of virus type is shown in table 1. Nineteen (61%) of the strains were of HSV-1, as shown by virus polypeptide and restriction enzyme analysis. Analysis of the relationship between the virus type and the nature

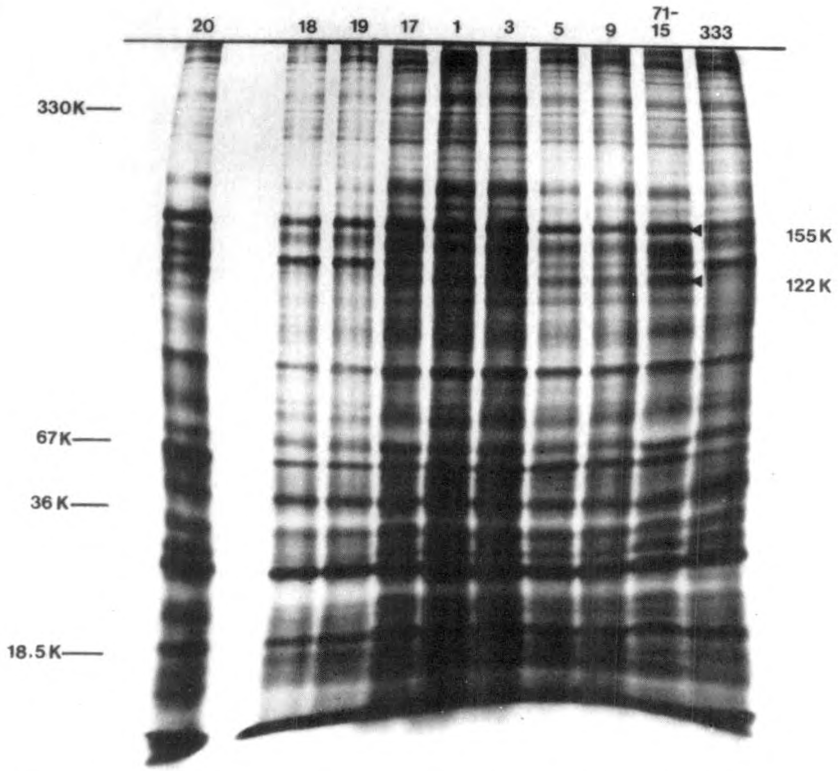


FIGURE *Sodium dodecyl sulphate polyacrylamide gel electrophoresis showing two strains of HSV-2 (18 and 19), six strains of HSV-1 (20, 17, 1, 3, 5, and 9), and the laboratory strains of HSV-1 (71-15) and HSV-2 (333). The position of the standard proteins of known molecular weight (thyroglobulin 330 K, bovine serum albumin 67 K, lactate dehydrogenase 36 K, and ferritin 18·5 K) are shown together with the major capsid polypeptide of HSV-1 (molecular weight 155 K) and the highly labelled band of molecular weight 122 K used in typing the isolates.*

of the genital lesion showed no apparent correlation; however, most of the isolates from men with primary infections were type 1. The distribution of types among primary and recurrent infections is shown in table II. There was no marked difference between the incidence of HSV-1 in primary (14/22) and recurrent (5/9) infections.

Discussion

Herpes genitalis is a disease which has increased in incidence in recent years; the infection is traditionally held to be associated with HSV-2, and this virus serotype is also considered an aetiological agent of carcinoma of the cervix.[17] The results of the present study do not confirm the marked association of genital herpes with the HSV-2 serotype reported by other workers. Thus, HSV-1 was recovered from genital sites with a much greater frequency than has previously been supposed and was the agent of both primary and recurrent genital infections in both sexes.

All 14 patients with primary HSV-1 infections admitted orogenital contact compared with four of eight of those with HSV-2 infections. Oro-genital contact was admitted less frequently by those with recurrent genital herpes. Patients with HSV-1 isolates admitted to fewer partners in the month before their attendance than did those with HSV-2 isolates. Fourteen of the 19 (74%) patients from whom HSV-1 was isolated admitted a single partner only compared with five of the 12 (42%) with HSV-2 isolates. In one instance HSV-1 was isolated from the cervix of a woman who had presented as the contact of a man who had previously been seen with primary genital herpes; in this case transmission was probably from the female patient's oral herpes via oro-penile contact, with subsequent transmission of virus to the cervix from the primary penile infection. The high incidence of genital infections with HSV-1 in this

TABLE I *Incidence of HSV-1 and HSV-2 isolated from a series of 31 patients*

Patient No (sex)	*Age (years)*	*HSV type**	*Restriction endonuclease†*
1 (M)	32	1	EcoRI, Hind III
2 (M)	27	1	Hind III
3 (F)	27	1	Hind III
4 (M)	25	1	Hind III
5 (M)	19	1	Hind III
6 (M)	22	1	Hind III
7 (M)	33	1	EcoRI, Hind III
8 (F)	23	1	ND
9 (M)	35	1	EcoRI, Hind III
10 (F)	16	1	EcoRI, Hind III
11 (F)	36	1	SmaI, Hind III
12 (F)	20	1	ND
13 (M)	19	2	Hind III
14 (M)	35	1	EcoRI, Hind III
15 (M)	51	2	EcoRI, Hind III
16 (F)	36	2	EcoRI, Hind III
17 (F)	16	1	EcoRI, Hind III
18 (M)	22	2	Hind III
19 (F)	17	2	ND
20 (F)	17	1	Hind III
21 (F)	17	2	Hind III
22 (M)	18	1	Hind III
23 (F)	20	2	ND
24 (F)	17	1	ND
25 (F)	42	2	EcoRI, Hind III
26 (F)	20	2	EcoRI, Hind III
27 (F)	26	1	ND
28 (F)	24	2	Hind III
29 (F)	25	2	EcoRI, Hind III
30 (F)	22	2	Hind III
31 (F)	22	1	EcoRI, Hind III

*Virus type as determined by analysis of infected cell polypeptides
†Restriction endonuclease used to confirm virus type.
ND = not done

TABLE II *Incidence of primary and recurrent infections with HSV*

	Primary infections		*Recurrent infections*		*Total*
HSV	*Men*	*Women*	*Men*	*Women*	*(%)*
Type 1	6	8	3	2	19 (16·3)
Type 2	1	7	2	2	12 (38·7)

group of patients may be the result of increasing acceptability and practice of oro-genital contact. This may be one factor which could explain why genital herpes infections appear to be increasing at a much greater rate than other sexually transmitted diseases. Transmission of HSV-1 by oro-genital contact may also explain the occurrence of herpes genitalis within sexually stable relationships where there have been no other partners.

The high frequency of HSV-1 isolation found in this group of patients has important implications if the results are confirmed in other centres. Firstly, the susceptibility of the two serotypes to treatment with antiviral agents varies; in general HSV-1 is more susceptible to antiviral drugs than HSV-2.[18 19] Therefore, in the assessment of antiviral compounds, reference must be made to the virus serotype since this may influence the results. Secondly, the proposed development of an HSV-2 vaccine for the control of herpes genitalis may have to be reconsidered should HSV-1 be shown to be an important aetiological agent of genital infection. Thirdly, the possible importance of HSV-2 as an aetiological agent of carcinoma of the cervix has been extensively reviewed[13 17]; but the present findings suggest that a similar role for HSV-1 should be investigated.

The work reported in this paper was supported by a grant from the Yorkshire Cancer Research Campaign.

References

1. Nahmias AJ, Starr SE. In: *Infectious Diseases* Hoeprich PD, ed. London: Harper and Row, 1979;726-35.
2. Nahmias A, Dowdle W. Antigenic and biologic differences in *Herpesvirus hominis. Prog Med Virol* 1968;**10:**110-59.
3. Dowdle W, Nahmias A, Harwell R, Pauls F. Association of antigenic type of *Herpesvirus hominis* to site of viral recovery. *J Immunol* 1967;**99:**974-80.
4. Smith IW, Peutherer JF, Robertson DHH. Virological studies in genital herpes (letter). *Lancet* 1976;ii:1089-90.
5. Kaufman RL, Rawls WE, Dixon R, Young RL. Clinical features of herpes genitalis. *Cancer Res* 1973;**33:**1446-51.
6. Kawana T, Kawaguchi T, Sakamoto S. Clinical and virological studies on genital herpes. *Lancet* 1976;ii:965.
7. Wheeler CE jun, Briggaman RA, Henderson RRJ. Discrimination between two strains (types) of herpes simplex by various modifications of the neutralization test. *J Immunol* 1969;**102:**1178-92.
8. Nahmias A, Chiang W, Del Buano I, Duffey C. Typing of *Herpesvirus hominis* strains by indirect immunofluorescent technique. *Proc Soc Exp Biol Med* 1969;**132:**386-90.
9. Cassai EN, Sarimento M, Spear PG. Comparison of the virion proteins specified by herpes simplex virus type 1 and 2. *J Virol* 1975;**16:**1327-31.
10. Powell KL, Mirkovic R, Courtney RJ. Comparative analysis of polypeptides induced by type 1 and type 2 strains of herpes simplex virus. *Intervirology* 1977;**3:**13-29.
11. Wilkie NM. Physical maps for herpes simplex virus type 1 DNA for restriction endonucleases. Hind III, Hpa-I and X-bad. *J Virol* 1976;**20:**222-33.
12. Skare J, Summers WP, Summers WC. Structure and function of herpes genomes: I Comparison of five HSV-1 and two HSV-2 strains by cleavage of their DNA with EcoRI restriction endonuclease. *J Virol* 1975;**15:**726-32.
13. Cortini R, Wilkie NM. Physical maps for HSV type 2 DNA with five restriction endonucleases. *J Gen Virol* 1978;**39:**259-80.
14. Lonsdale DM, Brown SM, Subak-Sharpe JH, Warren KG, Koprowski J. The polypeptide and DNA restriction enzyme profiles of spontaneous isolates of herpes simplex virus type 1 from explants of human trigeminal, superior cervical and vagus ganglia. *J Gen Virol* 1979;**43:**151-71.
15. Laemelli IK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;**214:**1334-6.
16. Lonsdale DM. A rapid technique for distinguishing herpes simplex type 1 and type 2 by restriction enzyme technology. *Lancet* 1979;i:849-50.
17. Royston I, Aurelian L. The association of genital herpes with cervical atypia and carcinoma *in situ. Am J Epidemiol* 1970;**91:**531-40.
18. De Clercq E, Descamps J, De Somer P, Barr PJ, Jones AS, Walker RT. (E)-5-(2-bromovinyl)-2′ deoxyuridine: potent and selective anti-herpes agent. *Proc Natl Acad Sci (USA)* 1979;**76:**2947-51.
19. Park A, Pavan-Langston D, Hettinger ME, *et al.* Topical therapeutic efficacy of 9-(2-hydroxymethyl) guanine and 5′-iodo-5′-amino-2′, 5′dideoxyuridine on oral infection with herpes simplex virus in mice. *J Infect Dis* 1980;**141:**575-9.

Br J Vener Dis 1982;**58:**48-51

Confirmation of herpes simplex virus type 2 infections in herpes-like genital lesions by a simple complement-fixation test

MINAS ARSENAKIS AND JOHN T MAY

From the Department of Microbiology, La Trobe University, Bundoora, Victoria, Australia

SUMMARY The presence of complement-fixing antibody to an early herpes simplex virus type 2 (HSV-2) antigen (the AG-4 antigen) was correlated with HSV-2 infection in the sera of patients with genital herpes. Eighty-eight per cent of sera taken two weeks after clinical diagnosis of a primary or recurrent herpes infection in patients, confirmed to have HSV-2 by virus isolation and typing, contained the anti-AG-4 complement-fixing antibody. None of the patients with genital HSV-1 had the antibody, and only 9% of controls or patients with facial HSV-1 infection had positive results for the antibody. This correlation was used to identify genital HSV-2 infections when either no virus sample had been taken or when virus isolations had been unsuccessful. Thus, a simple complement-fixation test can confirm an HSV-2 virus infection without isolation of the virus from the herpetic lesion.

Introduction

Two types of herpes simplex virus have been isolated from genital lesions. While herpes simplex type 2 (HSV-2) is the type most commonly found in ulcerative genital lesions (ranging from 60% to 90% depending on the population under study)[1 2] herpes simplex type 1 (HSV-1) has also been identified in genital lesions.[1 2] Isolation of the herpes simplex virus from the lesion is usually followed by typing by a variety of different methods, such as virus neutralisation,[3] immunoperoxidase methods,[4] thymidine kinase induction,[2] plaque morphology,[5] DNA restriction endonuclease analysis,[6] and others.[7] The unequivocal identification of HSV-2 type antibody in patients' sera has been difficult because of the cross-reaction between HSV-1 and HSV-2 antibody and the high incidence of antibody to type 1 virus in the community.[8] This problem may be overcome to some extent if a type-specific HSV-2 glycoprotein is used as the test antigen or if extensive absorption of sera with HSV-1-infected cells is performed.[9]

We report here that a simple complement-fixation test with a crude four-hour HSV-2-infected HEP-2 cell extract (containing the immediate early AG-4 antigen) can be used to distinguish rapidly between HSV-1 and HSV-2 infections. We have also used this simple test to confirm clinically diagnosed genital herpes as an HSV-2 infection.

Materials and methods

CELLS AND VIRUSES

Human epidermoid carcinoma (HEP-2) cells and HSV-2 strain G used to make the AG-4 antigen came from stocks held at the microbiology department, La Trobe University, Melbourne. (Both are also available from the American type culture collection, Rockville, Maryland, USA.) HSV-2 was propagated and titrated by plaque assay on HEP-2 cells maintained in Eagle's minimal essential medium (EMEM) supplemented with 10% newborn-calf serum (NBC) and 100 μg/ml neomycin.

BLOOD SAMPLES

Blood samples were obtained from 64 patients (40 women and 24 men, aged 18-30 years) with genital herpes-like lesions (from either Dr D Bradford of the Communicable Diseases Centre, Melbourne, or the physicians at La Trobe University Health Centre, Melbourne). Samples were also obtained from a control group of 23 patients, matched for age and sex distribution, without past or present HSV-2 infection. Blood was collected as soon as possible from these sources (usually within an hour) and sera harvested after storage of the blood overnight at 4°C.

Address for reprints: Dr J T May, Department of Microbiology, La Trobe University, Bundoora, Victoria 3083, Australia

Accepted for publication 9 September 1981

PREPARATION OF AG-4 ANTIGEN

HEP-2 cells were grown in Roux bottles in a humidified 5% CO_2 atmosphere at 37°C, and infected with HSV-2 strain G at a multiplicity of infection of 0·7 plaque-forming units (pfu)/cell for AG-4 preparation. The virus was adsorbed for one hour at 37°C and the cells then overlaid with EMEM supplemented with 1% newborn-calf serum and 100 μg/ml neomycin and incubated for a further four hours at 37°C. The medium was then removed, and the cells scraped off in sterile barbitone-buffered saline (BBS) and washed three times. The final cell pellet was resuspended in 0·7 ml of BBS per 1×10^7 cells, frozen and thawed four times, centrifuged at $1600 \times g$ for 15 minutes to remove cell debris, and stored at −70°C until used. Control antigen (AG-H) was prepared in the same way with mock-infected HEP-2 cells. The antigen concentration was adjusted to 2·0 mg/ml of protein.[10]

COMPLEMENT FIXATION

The technique was performed essentially as described.[10] The test was carried out in polystyrene tubes (Disposable Products Pty Ltd, Salisbury, S Australia). Guinea-pig complement, haemolysin, and sheep red blood cells (SRBC) were obtained from the Commonwealth Serum Laboratories, Melbourne, Australia. The test sera were diluted 1/4 in BBS and inactivated by heat at 56°C for 30 minutes. For the test, 0·1 ml of diluted serum, 0·1 ml of antigen or BBS, and 0·2 ml (2 units) of complement were incubated at 4°C for 17 hours. The tubes were then warmed at 37°C for 10 minutes, and 0·1 ml of BBS, 0·1 of haemolysin (2 units), and 0·1 ml of 2% SRBC (5×10^7cells/ml) added. After 60 minutes' incubation at 37°C the results were read. In all tests one standard positive and one standard negative control serum were included. The reaction was considered positive for AG-4 if 10% or more complement was fixed with AG-4 than with the control AG-H preparation. Also included was a set of standards representing the range 0-100% complement fixation. In every case in this study, no complement was ever fixed with the control AG-H preparation, although in previous studies some AG-H reactivity was observed.[10]

Each serum sample was tested for activity against AG-4 and AG-H together with a control serum to establish any anticomplementary activity. All test samples were run in duplicate in at least three separate experiments. For routine purposes a 1/4 dilution of sera was used to measure the presence of AG-4 antibody but some sera can be diluted further.

VIRUS ISOLATION AND TYPING

Virus samples were usually collected from herpes-like lesions with a sterile cotton applicator and placed in transport media by the physicians at the time the patients attended with lesions. The samples were forwarded to the Fairfield Infectious Disease Hospital, Melbourne, for isolation and typing by a standard virus neutralisation method.[3] Essentially, the herpes virus isolate was initially grown and isolated in human embryo lung (HEL) cells. The virus (100 TCD_{50}) was then added to microtitre plate wells (Lindbro tissue culture, Flow Laboratories, Australia) together with diluted antisera to either HSV-1 (MacIntyre) or HSV-2 (MS).[10] The cytopathic effect of residual herpes virus infectivity was determined on HEL cells. The antisera used in these virus neutralisation tests were prepared in rabbits by repeated injection of HSV-1 or HSV-2 over a four-year period. At the dilutions used (usually 1/25) the anti-HSV-1 sera neutralised both HSV-1 and HSV-2 viruses while the anti-HSV-2 sera neutralised only HSV-2 viruses. The results of the herpes simplex virus typing reached the physicians about four weeks after the sample was sent. Only about 4% of genital herpes isolates were typed as HSV-1 by Fairfield Infectious Disease Hospital (K Hayes, personal communication). The results of these typing tests were available to us only after the anti-AG-4 antibody titres had been determined.

Results

TIME OF ANTIBODY DETECTION

The time-course appearance of complement-fixing antibody to AG-4 in recurrent HSV-2 and in primary HSV-1 and HSV-2 infections in patients with genital herpes is shown in the figure. Over the five-week period no anti-AG-4 complement-fixing antibody was detected in the patients with genital HSV-1 infections. The antibody was usually detected two weeks after a primary infection of HSV-2 and was still present five weeks later. On the other hand, in

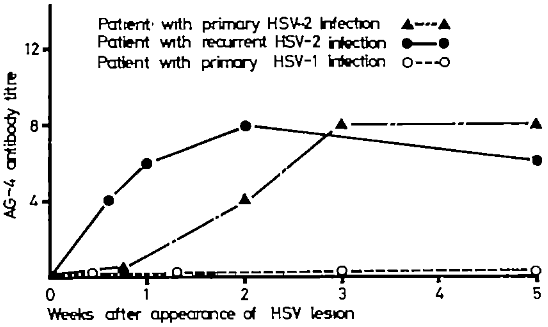


FIGURE *Appearance of anti-AG-4 complement-fixing antibody in the sera of three patients with herpes infection.*

50% of patients with recurrent HSV-2 infections (figure) the anti-AG-4 complement-fixing antibody could be detected at four days and increased to a maximum titre at two weeks after the lesions appeared. After this time, in unusual recurrent cases, the complement-fixing antibody titre may rise to as high as 1/64 or may fall rapidly.[11] Thus, the best time to take sera for the AG-4 complement-fixation test, for both recurrent and primary HSV-2 infections, is at two weeks after the appearance of lesions.

FREQUENCY OF ANTIBODY DETECTION

The frequency of appearance of anti-AG-4 complement-fixing antibody in a variety of patients with herpes-like lesions is shown in the table. The sera tested were taken 14 days after lesions appeared (although from most patients we had more than one serum sample at different time periods). In 29 of 33 (88%) patients with HSV-2 infections confirmed by virus isolation and neutralisation the antibody was present in their sera (at titres ranging from 1/4-1/64, mean titre 1/8; only three patients had titres >1/32). Sera with complement fixation at <1/4 were considered negative; those in controls that were positive had titres of 1/4-1/8.

In the genital HSV-1 infections no antibody was detected and in both the patients with facial HSV-1 and the controls only 9% of sera contained the antibody. Occasionally swabs from the herpes-like lesions yielded a virus that was not typed as herpes simplex and these patients did not have the antibody. When sera were taken from patients with genital herpes-like lesions, and no isolation of virus attempted, 82% contained anti-AG-4 complement-fixing antibody. The anti-AG-4 antibody titres were compared with the virus typing data only after the former were known.

If a similar extract of HEP-2 cells infected with HSV-1 for four hours (similar to the AG-4 preparation—that is, multiplicity of infection at 0·7 pfu/cell) was used in the test no complement fixation was detected with sera from patients with either HSV-2 or HSV-1 infections. If the HSV-1 infection rate was greater than 3 pfu/cell or the infection was allowed to proceed for 18 hours rather than four hours, however, most of the 14 days' HSV-2 sera (approximately 90%) would react with the HSV-1 extract in the complement-fixation test. This was probably because of the cross-reaction of HSV-1 antibody (HSV-neutralising antibody increases by twofold or threefold after infection[11]) with the late HSV-1 antigens.

Discussion

We have found that the antigen that patients with HSV-2 infections react to in HSV-2 four-hour-infected HEP-2 cells is an immediate early protein of 160 000 molecular weight.[11] This antigen has previously been used to detect anti-AG-4 complement-fixing antibodies in patients with cancer of the cervix.[10 12] We have found that it would also detect AG-4 antibodies in patients infected with HSV-2[10 11] but not in those infected with HSV-1.[10] Detection of the anti-AG-4 complement-fixing antibody in HSV-2 patients is, however, dependent on the time at which the sera are taken after recurrence or primary herpetic infection (figure). When sera are taken 14 days after the appearance of the herpes-like lesion, or sometimes earlier in recurrent episodes, this simple complement-fixation test can detect whether the lesion is due to either HSV-2 or HSV-1. Sera taken at the time of presentation with HSV-2 lesions were positive for the antibody only in some recurrent cases, but once the antibody appeared (in most cases) it was found to persist for six months.[11] Decline in the antibody titres has been found as early as four weeks[11] and in other recurrent cases it has persisted for at least 18 months. Thus it is possible in most cases to repeat or perform the complement-fixation test more than two weeks after the appearance of lesions.

Our results suggest that the sera of 88% of the patients with HSV-2 infections (diagnosed by virus isolation and typing by a neutralisation method)

TABLE *Frequency of anti-AG-4 complement-fixing antibodies in the various patient groups*

Patient group	*No tested*	*Positive results for AG-4 antibody*	
		No	*%*
Confirmed genital HSV-2	33	29	88
Confirmed genital HSV-1	4	0	0
Non-genital facial HSV-1	12	1	8·3
Herpes-like lesions but no virus isolation attempted (or virus sample not infectous)	11	9	82
Herpes-like lesions (virus other than herpes isolated*)	4	0	0
Controls (no HSV infection)	23	2	8·7

* Usually not identified other than as non-herpes virus. (Adenoviruses are occasionally isolated from the genital tract.[13])

contained the anti-AG-4 complement-fixing antibody. It is unlikely that any herpes typing technique is completely unambiguous,[3] although restriction technology has claimed to be so.[6] This complement-fixation test is simple and the results are obtained quickly and do not require virus isolation. Moreover, the test was not interfered with by the presence of HSV-1 antibody in the sera, a problem occurring with most serological tests,[1] and could be used well after the initial appearance of the herpes lesions. The anti-AG-4 complement-fixation test was found to be most useful when either no virus isolation was attempted at the time of examination or it was unsuccessful in confirming that the genital lesion was caused by HSV-2 and not HSV-1.

We wish to acknowledge the assistance of Dr D Bradford, the Communicable Diseases Centre, and the physicians at La Trobe University's Health Centre for supplying sera from herpes-infected patients. This work was supported by a grant from the Australian Research Grant Committee.

References

1. Kaufman RH, Gardner HL, Rawls WE, Dixon RE, Young RL. Clinical features of herpes genitalis. *Cancer Res* 1973; **33**:1446-51.
2. Kalinyak JE, Fleagle G, Docherty JJ. Incidence and distribution of herpes simplex virus type 1 and 2 from genital lesions in college women. *J Med Virol* 1977; **1**:175-81.
3. Kennett M, Donaldson A, Lewis FA. Herpes simplex. *In: Annual Report of Fairfield Hospital to the World Health Organisation.* Melbourne: Fairfield Hospital, 1977; 13.
4. Benjamin DP. Rapid typing of herpes simplex virus strains using the indirect immunoperoxidase method. *Appl Microbiol* 1974; **28**:568-71.
5. Ejercito PM, Kieff ED, Roizman B. Characterisation of herpes simplex virus strains differing in their effects on social behaviour of infected cells. *J Gen Virol* 1968; **2**:357-64.
6. Lonsdale DM. A rapid technique for distinguishing herpes simplex virus type 1 from the type 2 by restriction-enzyme technology. *Lancet* 1979; **i**:849-52.
7. Halliburton IW, Hill EA, Russell J. Identification of strains of herpes simplex virus by comparison of the density of their DNA using the preparative ultra-centrifuge. *Arch Virol* 1978; **48**:157-68.
8. Plummer G. A review of the identification and titration of antibodies to herpes simplex virus type 1 and 2 in human sera. *Cancer Res* 1973; **33**:1469-76.
9. Dreesman, GP, Matson DO, Courtney RJ, Adam E, Melnick JL. Detection of herpesvirus type-specific antibody by a macro-solid phase radioimmunometric assay. *Intervirol* 1979; **12**:115-9.
10. Arsenakis M, Georgiou GM, Welsh JK, Caughi MN, May JT. AG-4 complement-fixing antibodies in cervical cancer and herpes-infected patients using local herpes simplex virus type 2. *Int J Cancer* 1980; **25**:67-71.
11. Arsenakis M, May JT. Studies on complement-fixing antibody to the AG-4 antigen in herpes simplex virus type-2 infected patients. *Infect Immun* 1981; **33**:22-8.
12. Aurelian L, Schumann B, Marcus RL, Davis HJ. Antibody to HSV-2 induced tumor-specific antigens in serums from patients with cervical carcinoma. *Science* 1973; **181**:161-4.
13. Harnett GB, Newnham WA. Isolation of adenovirus type 19 from the male and female genital tracts. *Br J Vener Dis* 1981; **57**:55-7.

Br J Vener Dis 1982;**58**:52-3

Aseptic meningitis and herpes simplex proctitis

A case report

W A ATIA,* C S RATNATUNGA,* C GREENFIELD,* AND S DAWSON†

*From the *Marlborough Clinic and Medical Unit, Royal Free Hospital; and the †Praed Street Clinic, St Mary's Hospital, London*

SUMMARY A male homosexual patient developed an aseptic meningitis during the course of acute ano-proctitis due to herpes simplex virus type 2 (HSV-2). Aseptic meningitis (sometimes proved to be due to HSV-2) has been associated with genital but not with anal herpes. This sexually transmissible virus should be considered when patients with aseptic meningitis are investigated.

Introduction

The reported incidence of genital infection with the herpes simplex virus (HSV) seems to be increasing.[1] Various neurological syndromes have been described in association with genital herpes.[2 3] We report a patient who developed an aseptic meningitis during the course of acute herpetic ano-proctitis. This complication of herpes proctitis, although suspected,[4] has not been reported previously.

Case report

A 23-year-old man attended the Praed Street Clinic, St Mary's Hospital, London, on 16 June 1980 with a "sensation of swelling in the back" for one day. He had had casual passive homosexual contact four days previously.

INITIAL INVESTIGATIONS AND TREATMENT

On examination he had an inflamed rectal mucosa and purulent discharge. Routine investigations for sexually transmitted disease were performed. Microscopy showed polymorphonuclear cells in smears from the discharge containing intracellular Gram-negative diplococci. Ampicillin 3·5 g and probenecid 1 g were, therefore, given in a single dose. Culture results were negative for gonococci.

Two days later he reattended complaining of increasing anal discomfort, pain on defecation, some hesitancy of micturition, and hyperaesthesia in the back of his thighs. On examination there was bilateral tender enlargement of the inguinal lymph nodes. Multiple clusters of herpetiform vesicles were seen around the anus. Culture of samples from these vesicles later grew the herpes simplex virus (untyped). He was given co-trimoxazole tablets two twice daily for one week and an analgesic. He did not give any previous history of herpes infection.

COURSE OF ILLNESS

On 21 June 1980 the patient was admitted to the Royal Free Hospital with symptoms of malaise, headache, photophobia, hesitancy of micturition, and pain radiating down his legs. His temperature was 38°C, pulse rate 93/min, and he had signs of meningism. There was acute localised tenderness over the region of a lumbosacral spina bifida occulta, which he was known to have, but there was no neurological deficit. The perianal lesions had healed, but proctoscopy showed an inflamed ulcerated mucosa.

LABORATORY INVESTIGATIONS

Lumbar puncture was performed and showed the following results: cerebrospinal fluid clear, pressure 160 mm; WBC 560 × 10^6/l (560/mm^3) (90% lymphocytes, 10% leucocytes); RBC 100 × 10^6/l (100/mm^3); glucose 1·5 mmol/l (blood 6·5 mmol/l); and protein 0·7 g/l (normal value 0·4 g/l).

Cultures were bacteriologically sterile, but the CSF was not cultured for herpes virus. Full blood count and differential count, electroencephalogram, and radiographs of the chest were normal. Repeated Venereal Disease Research Laboratory and *Treponema pallidum* haemagglutination tests gave negative results.

VIRAL STUDIES

Rectal swabs taken on 26 June grew herpex simplex virus type 2. Bacterial and viral cultures of throat

Address for reprints: Dr W A Atia, Department of Venereology, Royal Northern Hospital, Holloway Road, London N7 6LD

Accepted for publication 8 July 1981

swabs gave negative results. Serum antibody titres for cytomegalovirus, mumps S, and mumps V were very low.

Serum herpes virus complement-fixing antibody titres were 1/64 on 26 June 1980 and on 22 July 1980.

MANAGEMENT

The patient was kept under observation and managed conservatively. His temperature returned to normal on the second day. He made an uneventful recovery and was discharged home after six days. He was seen as an outpatient four weeks later and there was no evidence of recurrence.

Discussion

Three days before admission this patient had symptoms suggestive of sensory root involvement. He then developed an aseptic lymphocytic meningitis during the course of active herpetic ano-proctitis. HSV was recovered from ano-rectal swabs both before the onset (untyped) and during recovery (type 2) from meningitis.

HSV accounts for only a small proportion of cases of aseptic meningitis. Active genital herpes—usually a primary attack—coexists with only about one-third of the reported cases of herpes meningitis.[5] Sexual transmission of HSV is known to occur in the absence of signs and symptoms of genital infection.[6] Furthermore, the virus can be recovered from the genital tract[7] or rectum[8] of such latent cases. A history of genital herpes should, therefore, be sought during investigation of the cause of aseptic meningitis. In the absence of such a history or other obvious aetiology the examination of appropriate ano-genital specimens (including prostatic fluid) for culture of HSV can be helpful, especially since a rise in serum herpes antibody titre may not appear during a recurrent attack.

Apart from epidemiological and theoretical considerations, the diagnosis of HSV as a cause of meningitis could have therapeutic implications in the near future should current evaluations of systemic acycloguanosine in the treatment of herpetic infections prove successful.

We thank Dr J W R Harris, Professor N McIntyre, and Dr D C McDonald Burns for encouragement and advice.

References

1. Academic Department of Genitourinary Medicine (Middlesex Hospital), Communicable Surveillance Centre, the Communicable Diseases Unit (Scotland). Sexually transmitted diseases surveillance 1978. *Br Med J* 1979;ii:1375-6.
2. Craig CP, Nahmias AJ. Different patterns of neurologic involvement with herpes simplex virus types 1 and 2: Isolation of herpes simplex virus type 2 from the buffy coat of two adults with meningitis *J Infect Dis* 1973;**127**:365-72.
3. Terni M, Caccialanza P, Cassai E, *et al.* Aseptic meningitis in association with herpes progenitalis. *N Engl J Med* 1971;**285**:503-4.
4. Oates JK, Greenhouse PRDH. Retention of urine in anogenital herpetic infection. *Lancet* 1978;i:691.
6. Sköldenberg B, Jeansson S, Wolontis S. Herpes simplex virus type 2 and acute aseptic meningitis. *Scand J Infect Dis* 1975;7:227-32.
6. Anonymous. Genital herpes. *Br Med J* 1980;i:1335-6.
7. Deardourff SL, DeTure FA, Drylie DM, *et al.* Association between *Herpesvirus hominis* type 2 and the male genitourinary tract. *J Urol* 1974;**122**:126.
8. Goldmeier D. Proctitis and herpes simplex virus in homosexual men. *Br J Vener Dis* 1980;**56**:111.

Br J Vener Dis 1982;**58**:54-5

Thiamphenicol in the treatment of chancroid in men

A S LATIF

From the City Health Department and the Department of Medicine, University of Zimbabwe Medical School, Salisbury, Zimbabwe

SUMMARY Thiamphenicol was used to treat 547 men with chancroid. An oral dose of 2·5g was given on the first day and a further dose of 1·25g a week later if the lesions had not healed. Eighty-seven (15·9%) patients defaulted from follow up and 23 (4·2%) had positive serological test results for syphilis. Of the remaining 437 patients, 27 (6·2%) failed to respond to treatment, 258 (59%) were cured after the single dose, and 152 (34·8%) required a second dose. The overall cure rate was 93·8%.

Introduction

Thiamphenicol is a broad-spectrum antibiotic, has a bacteriostatic action against a wide range of Gram-positive and Gram-negative bacteria and anaerobes, is closely related to chloramphenicol, and has few side effects. It has been used in the treatment of uncomplicated gonorrhoea in a single dose of 2·5g with high cure rates.[1] A single dose of 2·5g of thiamphenicol has been found to be effective against syphilis during the incubation period.[2]

Patients and methods

Patients included in the trial were men who presented voluntarily with chancroid to the Mbare Sexually Transmitted Diseases Clinic in Harare, Salisbury.

DIAGNOSTIC CRITERIA

The diagnosis of chancroid was made by the clinical finding of painful, non-indurated, necrotic ulcers, exudates of which showed no *Treponema pallidum* on darkground microscopy but showed typical slender Gram-negative bacilli in small chains and clusters. The Venereal Disease Research Laboratory (VDRL) test and the *T pallidum* haemagglutination assay (TPHA) were carried out on all patients at the time of the first visit and six weeks later. Of 1018 patients tested, 547 met the diagnostic criteria and were included in the study. Cultures for *Haemophilus ducreyi* and the Ito-Reenstierna intradermal tests were not carried out because of lack of facilities.

TREATMENT AND FOLLOW-UP

After the detection of *H ducreyi* in Gram-stained smears of ulcer material the patients were given 2·5g (10 capsules) of thiamphenicol to swallow at the clinic under supervision. They were asked to return after seven days and advised not to have sexual intercourse nor take any alcohol till the sores had healed. They were also advised on simple personal hygiene, told to wash the genital area thoroughly with soap and water at least twice a day, and warned not to apply any form of ointment locally.

If at the end of seven days the ulcers showed signs of healing and were no longer painful or tender on examination with a cottonwool-tipped swab the patient was given no further treatment but asked to return in a further week's time. If the ulcers were still painful the patient was given 1·25g of thiamphenicol to take at the clinic and was asked to return a week later.

If at the third visit the ulcers had healed or showed signs of healing no further treatment was given and the patient was asked to return after two weeks for examination. If, however, the ulcers had still not healed, treatment failure was assumed and the patient was given co-trimoxazole 800mg three times daily for seven days.

At the end of six weeks blood was taken for VDRL and TPHA tests. Exudates from ulcers were examined by darkfield microscopy, and Gram-stained smears of ulcer material were examined for *H ducreyi* at each visit.

Results

One thousand and eighteen patients with genital ulcers were examined. In 547 (53·7%) patients

Address for reprints: Dr A S Latif, 14 Viscount Bend, Ridgeview, Belvedere, Salisbury, Zimbabwe

Accepted for publication 23 September 1981

exudates from ulcers showed organisms resembling *H ducreyi.* Of these, 87(15·9%) defaulted from follow up (63 after the first visit and 24 after attending twice). Twenty-three (4·2%) had a posititive TPHA result, and of these, 18 also had a positive VDRL test result.

Inguinal buboes were present in 240 patients; in 225 (51·5%) they were unilateral and in 15 (3·4%) bilateral.

Of those followed up fully, 258 (59%) patients needed only the first dose of treatment and a further 152 (34·8%) were cured after the second dose. Twenty-seven (6·2%) patients failed to respond to treatment.

In the patients with buboes the size of the swelling had greatly subsided at the end of two weeks, although some induration was still palpable at the end of six weeks.

Discussion

Chancroid is the commonest form of STD seen among male patients attending STD clinics in Salisbury.[3 4] Patients with chancroid are usually treated with a seven-day course of tetracycline or intramuscular streptomycin. Prolonged courses of treatment are often necessary and hence patients commonly default. Single-dose regimens for the treatment of trichomoniasis, uncomplicated gonococcal urethritis, and early syphilis are now generally recommended.[5] No satisfactory single-dose treatment regimens are at present available for the treatment of chancroid. This trial suggests that two large doses of thiamphenicol given a week apart may be of considerable value in the treatment of this infection.

I wish to thank Mr Graham Wright and Mr George Dempster of DATLABS, Sinoia Street, Salisbury, for supplying Urfamycin capsules used in this trial.

References

1. Siboulet A. Minute treatment of gonoccal urethritis of the male, *Sem Ther* 1965; **41**:307.
2. Petzoldt D. Single-dose treatment of gonorrhoea with penicillin or thiamphenicol and effect on *Treponema pallidum* in experimental syphilis. *Br J Vener Dis* 1971; **47**;377.
3. Latif AS. Sexually transmitted disease in clinic patients in Salisbury, Zimbabwe. *Br J Vener Dis* 1981;**57**:181-3.
4. Bingham JS. Zimbabwe: land of hope and...*Br J Vener Dis* 1981;**57**:184-6.
5. Schofield CBS. *Sexually Transmitted Diseases,* 3rd ed. Edinburgh: Churchill Livingstone, 1979.

Br J Vener Dis 1982;**58**:56-8

International symposium on trichomoniasis: the Polish medical scene

R D CATTERALL*
From the Department of Genitourinary Medicine, Middlesex Hospital, London

The sixth International Congress of Protozoology was held in Warsaw from 5-11 July 1981. A satellite international symposium on trichomoniasis was held in Bialystok, a small industrial and university town in north-east Poland, from 13-15 July 1981. The symposium was organised by Professor Stefan Soszka, professor of obstetrics and gynaecology at the Medical Academy of Bialystok, Professor Bronislaw Hönigberg, professor of protozoology at the University of Massachusetts, Amhurst, USA, and Professor Miklos Müller, of the Rockefeller University, New York, USA.

International symposium on trichomoniasis

The opening ceremony took place in the Aula of the Medical Academy, which is housed in a beautiful 18th century château. Both the château, which was virtually undamaged during the second world war, and the extensive gardens have been maintained in excellent condition.

The delegates were welcomed by the dean of the Medical Academy and by Professor Stefan Soszka, who said how pleased he was to see so many doctors and scientists from Eastern Europe, Western Europe, and North America. Dr R D Catterall (London), president of the International Union against the Venereal Diseases and Treponematoses (IUVDT) and the official representative of the World Health Organisation, pointed out that a great deal of excellent scientific and clinical work had been carried out on trichomoniasis by workers in Eastern Europe. He expressed the gratitude of the delegates to the commission on trichomoniasis of the General Council of the Polish Parasitological Society and the Medical Academy in Bialystok for arranging the symposium in such beautiful surroundings and for their generous hospitality.

SCIENTIFIC AND CLINICAL PAPERS

At the first session Professor Bronislaw Hönigberg gave a detailed paper on the fine structure of *Trichomonas vaginalis* in relation to other trichomonads and Professor Miklos Müller reviewed the present state of knowledge of the functional morphology of *Trichomonas vaginalis*. After a lively discussion it was agreed that *Trichomonas vaginalis* is a phagocytic organism. Most of the ingested bacteria are destroyed in vacuoles within 15 to 20 minutes and experimental evidence shows that there is also direct killing of gonococci by trichomonads.

Professor Juri Teras (USSR) claimed to have isolated *Trichomonas vaginalis* from the bronchi and bronchial secretions of 80 out of 400 patients. Agglutination reactions were also positive in many cases and specific antibodies were detected in the sera of several patients. The role of orogenital sex in this finding was discussed and the question of whether *Trichomonas vaginalis* or other trichomonads actually cause respiratory disease was debated during the discussion. Many of the delegates expressed doubt about the interpretation of the findings.

Several papers describing the biomorphology of round, multinucleated forms of *Trichomonas vaginalis* led to a detailed discussion about round cyst-like forms so frequently described by French workers. Strong doubts were expressed whether these round structures were really forms of *Trichomonas vaginalis* and there was scepticism about whether they ever developed into motile, flagellated, pathogenic forms either in vitro or in vivo.

Understanding of the metabolism of *Trichomonas vaginalis* has advanced considerably in recent years. The subject was fully reviewed by Dr David Linsted (England) and the mechanism of the development of resistance to metronidazole discussed. *Trichomonas vaginalis* is regarded as an aero-tolerant anaerobe but its redox carriers are readily destroyed by oxygen. The hydrosome was thought to play an important role in metronidazole resistance.

A complete review of the present state of the immunology and serology of human trichomoniasis was presented by Dr John Ackers (London). The role

---

*President, IUVDT

Address for reprints: Dr R D Catterall, Department of Genitourinary Medicine, Middlesex Hospital, London W1N 8AA

Accepted for publication 18 August 1981

of polymorphonuclear leucocytes in killing *Trichomonas vaginalis* and the importance of complement and C3 were considered and the production of local antibodies described. Dr R D Catterall (London) reviewed recent work on the epidemiology and diagnosis of human trichomoniasis from a clinical standpoint and Dr Michael Spence (Baltimore, USA) described the association of *Trichomonas vaginalis* and other sexually transmitted organisms in human genital infections.

FUTURE AREAS OF RESEARCH

The large number of papers on various experimental and clinical aspects of human trichomoniasis indicated the interest still taken in the subject, and it was agreed that many problems remained to be solved. These included: important aspects of the metabolism of *Trichomonas vaginalis* and other trichomonads; the reasons for the continuing high prevalence of infection throughout the world, despite effective curative treatment; the failure to detect the protozoon in the male sexual partners of infected women in most cases; the possible existence of rounded cyst-like forms and their clinical significance; and the development of resistance to trichomonacidal drugs. *Trichomonas vaginalis* provides a very satisfactory model for the study of other protozoa, especially their biochemistry and metabolism, and there are several areas where useful future research work is indicated.

It was suggested that the next international symposium on trichomoniasis should be held in Prague in 1983.

The Polish medical scene

Polish doctors base their practices on Western European, British, and North American medicine. Most specialised textbooks are in English, however, and keeping up to date with modern developments is difficult, since there are very few medical visitors from the West, and foreign medical journals are scarce and expensive. Although permission may be obtained to attend international meetings abroad, only one delegate from Poland is usually sent because of expense.

Health care is free for everyone in communist states in Eastern Europe and is based on hospitals much more than in the United Kingdom. The amount of care available to patients is rationed by the size and sophistication of the district hospitals and the scope of the services available in the teaching hospitals and main centres in Warsaw.

SERVICES FOR STDs

The service for patients with sexually transmitted diseases is undertaken by the department of dermatovenereology at the various hospital centres, as is traditional in European countries. At the Medical Academy in Warsaw, however, dermatology and venereology are separated. The Institute of Venereal Diseases is housed in the same building as the department of dermatology but is an independent unit. It is largely the brain-child of Professor Josef Towpik, who is well known to members of the International Union against the Venereal Diseases and Treponematoses and who took an active part in the travelling seminar which visited the United States in 1971. He has been a member of several scientific groups of the World Health Organisation and was recently the president of the Polish Medical Association; he has now retired from medical practice. His successor as head of the Institute of Venereal Diseases of the Warsaw Medical Academy is Dr Tomasz Mroczkowski.

The institute occupies part of an old hospital building with spacious lofty rooms. There is both an outpatient section and beds in the wards. Patients with STDs wait in the corridors together with those with skin diseases, and there is no appointment system. The staff have regular clinical meetings and discussions.

The venereological laboratories are some distance away from the institute and are directed by a microbiologist, associate professor Dr Danuth Weyman-Rzucidto. Roiron's medium is used for cultures for gonorrhoea, and Roiron's TV medium for growing *Trichomonas vaginalis*, and Sabouraud's medium for *Candida;* a culture service for *Chlamydia trachomatis* has just been started.

The routine serological tests for syphilis performed on all sera are the Venereal Disease Research Laboratory (VDRL) and fluorescent treponemal antibody-absorption (FTA-ABS) tests. In patients showing a positive result an IgM-FTA test is usually performed. The *Treponema pallidum* haemagglutination assay (TPHA) is also available, and the *T pallidum* immobilisation (TPI) test is sometimes performed in difficult cases.

The overall impression was of an active keen unit with high standards of patient care.

SOCIAL AND POLITICAL LIFE

It is very evident that the church is still the focal point of Polish life despite 35 years of rigid communist rule. The visit of Pope John Paul in 1979 brought about spontaneous mass meetings all over the country and was a major factor in the rapid and astonishing growth of the trade union Solidarity. Its impact can be seen everywhere in the form of signs, posters, display cases, and lapel badges. In the hospitals, in public buildings, and in the market places literature about free trade unions is openly displayed all over the country. Television programmes are remarkably outspoken and present the aims of Solidarity to the nation.

The principal objective of Solidarity is a Polish government which is responsive to the wishes of the people. In the health service its aims are to eliminate political influence in recruitment, to improve the medical curriculum, and to have a less rigid academic structure. Many Poles apparently remain communists but do not wish to see the continuation of the rigid Soviet-type leadership.

There are, however, serious shortages all over the country. Food is scarce and certain medicines and drugs unobtainable. Queues exist everywhere and are getting worse. The shops are empty of goods but the people still look well nourished and well dressed.

THE FUTURE

The country is bubbling with expectation and guarded optimism. People are very friendly to foreigners and want to talk; above all they want moral and personal support from the West. Personal relationships are very important to them and they are eager for contact with foreigners. Medical journals and books are highly prized and clinical exchanges and fellowships greatly appreciated, as are visits to Poland by Western doctors. In the face of mounting difficulties due to shortages in the hospitals, Polish doctors are faced with a testing time ahead and need the support of the profession everywhere in the world.

Br J Vener Dis 1982;**58:**59-61

Cell-mediated immunity in HBeAg-positive homosexuals with chronic liver disease

L VIOLA, I G BARRISON, F PARADINAS, J C COLEMAN, AND IAIN M MURRAY-LYON

From the Gastrointestinal Unit and the Departments of Histopathology and Virology, Charing Cross Hospital and Medical School, London

SUMMARY Chronic liver disease developing after infection with the hepatitis B virus may be due to impairment of cell-mediated immunity (CMI). In a study of CMI by the lymphocyte transformation test to two mitogens and hepatitis B surface antigen (HBsAg) the response to the mitogens, phytohaemagglutinin (PHA) and purified protein derivative (PPD), was normal in all of 15 male homosexuals with HBsAg-positive chronic liver disease, indicating a normal non-specific cellular immune response. By contrast, only one of the 15 patients showed a response to HBsAg, which may explain the high prevalence of chronic HBsAg carriage in these patients.

Introduction

About 5% of homosexual men are hepatitis-B-surface-antigen-(HBsAg)-positive and many have serious asymptomatic chronic liver disease.[1] The pathogenetic events responsible for hepatocellular injury in chronic hepatitis B virus (HBV) infections have not yet been identified. Because the virus does not appear to be cytopathic for infected liver cells attention has been focused on host-determined genetic and immunological factors, particularly cell-mediated immunity (CMI). The presence of the HBsAg in the serum of many of these patients may indicate a generalised impairment of host immune response to the HBV.[2] Studies of CMI using lymphocyte transformation to a range of mitogens including HBsAg have not given consistent results,[3] but most of these reports do not take account of the HBe antigen/antibody status, except for one recent investigation of non-specific CMI.[4]

The aim of this study was to investigate the CMI response to different mitogens, including purified HBsAg, in a group of Caucasian homosexual men all of whom had high titres of HBsAg in serum, were hepatitis B e antigen (HBeAg)-positive, and had abnormal liver histology.

Address for reprints: Dr I M Murray-Lyon, Gastrointestinal Unit, Charing Cross Hospital (Fulham), Fulham Palace Road, London W6 8RF

Accepted for publication 13 August 1981

Patients and methods

Fifteen Caucasian male homosexuals (mean age 29·7 years) were studied. All were sexually active with 30-50 different contacts a year and were asymptomatic chronic HBsAg-carriers of between 12 and 96 months' duration (table). All were HBeAg-positive and anti-HBe-negative by radioimmunoassay. None had any physical signs of liver disease, but all had abnormal results to liver function tests. None was receiving drug treatment.

As controls, we also studied six normal healthy HBsAg-negative and anti-HBsAg-negative subjects and six patients who had recently recovered from an attack of acute type-B hepatitis with return of normal liver function, clearance of HBsAg from serum, and conversion to anti-HBs.

SEROLOGY

The presence of HBsAg was determined by reversed passive haemagglutination (Hepa test, Wellcome). HBeAg and anti-HBe were detected by radioimmunoassay (Abbott).

HISTOLOGY

All 15 patients has at least one liver biopsy performed. Histological diagnoses (table) were chronic active hepatitis (10 patients) and chronic persistent hepatitis (five patients). The Shikata (orcein) stain for HBsAg in liver cells gave a positive result in each case.

TABLE *Clinical, virological, and immunological data on the 15 patients studied*

Age (years)	*Duration of HBsAg carriage (months)*	*HBsAg titre*	*Liver histology*	*Lymphocyte transformation**		
				PHA	*PPD*	*HBsAg*
27	16	1/128 000	CPH	+ + +	+ + +	−
38	13	1/64 000	CAH	+ + +	+ + +	−
37	19	1/64 000	CAH	+ + +	+ +	−
30	96	1/16 000	CAH	+ + +	+ + +	−
40	72	1/128 000	CPH	+ + +	+ +	−
40	36	1/16 000	CAH	+ + +	+ + +	−
19	15	1/128 000	CPH	+ + +	+ + +	−
18	15	1/64 000	CPH	+ + +	+ +	−
25	18	1/256 000	CAH	+ + +	+ + +	−
30	15	1/32 000	CAH	+ + +	+ + +	+
25	14	1/64 000	CAH	+ + +	+ +	−
28	14	1/32 000	CAH	+ + +	+ +	−
26	18	1/256 000	CAH	+ + +	+ + +	−
24	16	1/8000	CAH	+ + +	+ +	−
32	9	1/128 000	CPH	+ + +	+ +	−

*Difference between stimulated and control cultures: + + + = >10 000 cpm; + + = >5000 cpm; + = >1000 cpm; − = <1000 cpm.
CPH = chronic persistent hepatitis; CAH = chronic active hepatitis; PHA = phytohaemagglutinin; PPD = purified protein derivative; HBsAg = hepatitis B surface antigen.

LYMPHOCYTE TRANSFORMATION

The in-vitro lymphocyte transformation was measured by uptake of ^{3}H thymidine and recorded as counts per minute (cpm) above background counts. The mitogens used were phytohaemagglutinin (PHA, Wellcome Laboratories) in concentrations of 0·5-4μg/ml, purified protein derivative (PPD; Central Veterinary Laboratory) in concentrations of 0·1-100 μg/ml, and HBsAg from chimpanzee plasma (London School of Hygiene and Tropical Medicine) purified by caesium chloride gradient ultracentrifugation and used in concentrations of 0·6-80 μg/ml of protein. All concentrations of the mitogens were used for the patients and the controls. Each experiment was performed in triplicate and a single preparation of type AB serum used in all the cell cultures.

Mononuclear cells were separated from sterile heparinised blood by density gradient centrifugation as described by Boyum.[5] The cells collected at the interphase were washed three times with Hank's balanced salt solution (Flow Laboratories). Prepared lymphocytes were suspended at a final concentration of 10^6/ml in RPMI 1640 medium supplemented with 20% AB serum, L-glutamine, penicillin, and streptomycin. The cells were placed in microtitre plates and mitogens and antigen at different concentrations were added. The plates were incubated at 37°C in a moist 5% CO$_2$/95% air atmosphere for five days and pulsed with 0·5 μ Ci/well of ^{3}H thymidine (Radiochemical Centre, Amersham, England) for the last 18 hours; the cultures were then harvested on glass-fibre filter paper using an automatic cell harvester (Flow Laboratories) and ^{3}H thymidine uptake was measured on a β-liquid scintillation counter.

Results

The responses to the different mitogens are shown in the table. In every case there was a strongly positive response to PHA and PPD indicating normal T-cell function, and no significant difference in this response was evident between the patients and the controls. With purified HBsAg, however, a response occurred in only one of 15 patients, but three of the six controls who had recovered from acute type B hepatitis responded. The counts were between 5 000 and 10 000 cpm in two patients and more than 10 000 cpm in the third. None of the six healthy HBsAg-negative and anti-HBs-negative controls responded to this antigen.

Discussion

This study shows that in this well-defined group of male homosexual patients with high titres of serum HBsAg, detectable HBeAg, and abnormal liver function a normal non-specific cellular immune response to PHA and PPD occurred, despite a previous report to the contrary.[3] A specific CMI response to HBsAg develops in the recovery phase of acute type-B hepatitis in some, but not all, patients and there appears to be a specific defect in this response in asymptomatic healthy carriers. Conflicting results, however, have been obtained previously in patients with chronic HBsAg-positive liver disease.[6] This may have been due to the heterogeneous nature of the patients investigated. In the present study we have shown that more than 90% of the patients failed to respond to purified HBsAg.

This lack of response may explain the chronic carrier state and allow continued viral replication. If CMI has a role in the pathogenesis of the liver damage it does not seem to be directed against HBsAg; other viral antigens, such as hepatitis B core antigen (HBcAg) or host liver protein, may possibly be implicated.

L Viola is in receipt of a grant from the University of Buenos Aires, Argentina. I G Barrison is in receipt of a grant from the trustees of Charing Cross Hospital. We are grateful to Professor A J Zuckerman (London School of Hygiene and Tropical Medicine), for assistance and for supplies of purified HBsAg, and to Dr J L Fluker and Dr B A Evans for their continuing close co-operation.

References

1. Ellis WR, Murray-Lyon IM, Coleman JC, *et al.* Liver disease among homosexual males. *Lancet* 1979; i:903-5.
2. Vogten AJM, Summerskill WHJ, Gitnick GL, *et al.* Behaviour of e antigen and antibody during chronic active liver disease. Relation to HB antigen-antibody system and prognosis. *Lancet* 1976; ii: 126-8.
3. Edgington TS, Chisari VF. Immune responses to hepatitis B virus coded and induced antigens in chronic active hepatitis. In: Eddleston ALWF, Weber JCP, Williams R, eds. *Immune Reactions in Liver Disease.* Tunbridge Wells: Pitman Medical Ltd, 1979: 44-60.
4. Pastore G, Tursi A, Loria MP, Dentico P, Fera G, Schiraldi O. HBeAg/anti-HBe system and cell-mediated immunity in patients with HBsAg-positive chronic active hepatitis. *Digestion* 1980; **20**: 214-20.
5. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968; **21**: 77-88.
6. Frei CP, Desaules M, Krafft T. Cell-mediated immune response to purified HBsAg in HBV infections. In: Vyas NG, Cohen NS, Schmid R, eds. *Proceedings of the Second Symposium on Viral Hepatitis.* Philadelphia: The Franklin Institute Press, 1978: 267-73.

Br J Vener Dis 1982;**58:**62-5

Autogenous vaccine therapy for condyloma acuminatum

A double-blind controlled study

MICHAEL D MALISON, RODNEY MORRIS, AND LAWRENCE W JONES
From the Huntington Institute of Applied Medical Research, Pasadena, California, USA

SUMMARY In an attempt to substantiate claims that autogenous vaccine is an effective form of immunotherapy for condyloma acuminatum, a double-blind cross-over study was carried out on 34 patients, in which autogenous wart vaccine was compared with a placebo vaccine identically prepared from each patient's own normal skin. It was found that the duration of disease was an important determinant of curability in patients with condylomata acuminata ($P<0\cdot001$) and that when this factor was taken into account autogenous wart vaccine was not significantly more effective than the placebo ($P=0\cdot43$).

Introduction

Condyloma acuminatum is a contagious, unsightly, and often persistent problem seen by a wide variety of medical and surgical practitioners. Caused by the human papilloma virus (HPV), this disease represents the only known virally induced tumour in man,[1 2] and cases of malignant transformation—although rare—have been reported.[3] While definite causality has not been proved in these cases, the presence of this disease may identify a host of greater susceptibility to certain forms of cancer.[4]

Evidence has recently been published which differentiates the HPV associated with common warts from that of the genital lesions on the basis of viral DNA homology studies.[5] Antigenically, however, the differences are not so distinct.[6 7] At present, confusion still exists about the roles played by the various components of the immune system in the resolution of human wart disease. This confusion is perhaps a reflection of the underlying complexity of the problem, and only in the last few years has the immunology of human wart disease captured the interest of researchers as a potential model for the study of human tumour immunology.

Historically, however, the first attempted application of immunology to the problem of human wart disease occurred over half a century ago. Biberstein, in 1925, theorised that the remission of warts was somehow linked to the host's immune system.[8] In an attempt to stimulate the immune system he injected his patients with a crude wart extract and attributed the resulting high cure rate to an evoked immune response. A dispute in the literature ensued when Cormia[9] could not reproduce Biberstein's results in 1934. This, and the publication of excellent results in the treatment of condylomata with podophyllin in 1944 by Culp and Kaplan,[10] caused Biberstein's technique to be largely forgotten.

Powell revived Biberstein's "vaccination" technique for treating condylomata acuminata in 1970 and reported cures in 23 out of 24 patients with lesions refractory to conventional measures.[11] Powell suggested that the wart virus was relatively "insulated" from the immune system in the superficial layers of the epithelium of the wart and that systemic injection of the wart extract provided more direct exposure of the antigen resulting in enhanced immunological destruction of the remaining wart tissue. Others have reported similar encouraging results with autogenous vaccine therapy in the past decade; however, none of these studies has included a control population of patients.[12-16]

Given the capricious nature of wart disease, it seemed desirable to carry out a study to evaluate the effectiveness of autogenous vaccine therapy for condyloma acuminatum in which the bias from spontaneous remission and placebo effect, or both, could be separated.

Address for reprints: Dr R Morris, Huntington Institute of Applied Medical Research, 734 Fairmount Avenue, Pasadena, California 91105, USA

Accepted for publication 8 July 1981

Patients and methods

STUDY POPULATION

Thirty-four patients (23 male and 11 female) with condylomata acuminata in various locations (table I) were studied prospectively in a double-blind cross-over fashion. The duration of disease ranged from a few months to 35 years with a mean of 3·6 years. Twenty-one patients had had warts for less than a year, nine for 1-5 years, and four for more than five years. In 18 (53%) patients previous forms of conventional therapy, which included podophyllin, fulguration and simple excision, had failed; the remainder of the patients had not been treated previously (table II).

TABLE I *Distribution of condylomata acuminata by sex and location*

Patients	*Genital*	*Anal*	*Both*	*Total*
Male	8	7	8	23
Female	7	0	4	11

TABLE II *Previous methods of treatment for 18 patients with refractory lesions*

Treatment	*No of patients*
Podophyllin	17
Cryosurgery	5
Fulguration	4
Topical 5-fluorouracil	2
Simple excision	2
Combined therapies	8

Before enrolment in the study, patients were screened for gonorrhoea and syphilis. Three cases of gonorrhoea were detected and treated .

PREPARATION OF VACCINE

After informed consent had been obtained a sufficient quantity of wart tissue was excised (0·5-1·0 g) from each patient to produce an autogenous vaccine according to the method of Powell *et al.*[11] A specimen was submitted in each case for histological confirmation of the diagnosis. A similar quantity of normal skin was also excised from the non-dominant elbow region of each patient for the production of a placebo vaccine by an identical method, care being taken to prevent cross-contamination of specimens both at operation and in the laboratory.

Laboratory technique

Tissue was collected in sterile Hank's buffered saline solution containing 100 μg/ml gentamicin and transferred to the laboratory where it was either prepared immediately or stored frozen at −70°C for preparation the next day. Fresh or thawed tissue was minced with a sterile scalpel and placed in a centrifuge tube with sterile saline at a ratio of 9:1 (saline-to-tissue) (10% by weight). This mixture was then homogenised in a Ten-Broek tissue grinder and subsequently frozen and thawed four times in dry ice/alcohol and a 37°C water-bath. The resulting suspension was clarified by centrifugation at 5000 rev/min for 10 minutes. The supernatant was collected, inactivated by heating at 56°C for one hour, and recentrifuged at 10 000 rev/min for 10 minutes. The supernatant was collected as the vaccine, filtered through a 0·45 μ Millipore filter, and tested for sterility before administration. The final protein concentration of the vaccines varied considerably and ranged from 12 to 157 mg/ml with a mean of 57·3 mg/ml.

A limited number of vaccine preparations was centrifuged at 50 000 rev/min, the sediment stained with 2% phosphotungstic acid and examined by electron microscopy for the presence of viral-type particles. Several wart specimens were fixed, embedded in Epon 12, sectioned, and examined by electron microscopy for intranuclear virus-type particles (fig 1).

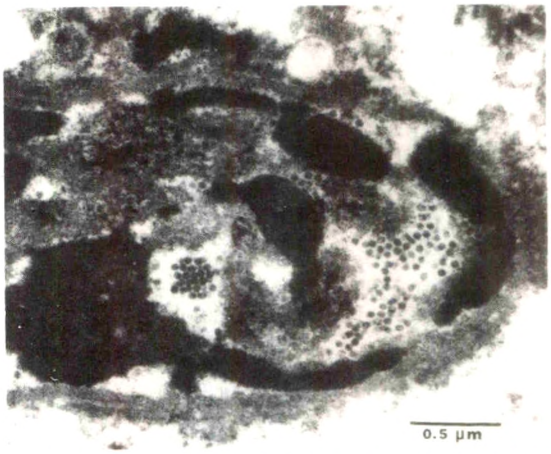


FIG 1 *Electron micrograph of intranuclear virus particles in a keratinocyte from a venereal wart.*

ALLOCATION OF THERAPY

A schematic diagram of the study plan is shown in fig 2. Patients were randomly selected by lottery to receive first either placebo or wart vaccine. The coded vaccines, identical in appearance, were administered as weekly subcutaneous injections of 0·5 ml over the deltoid region for six weeks.[16] All vaccines were stored frozen and thawed just before each administration. A positive response usually occurred before completion of the first series of

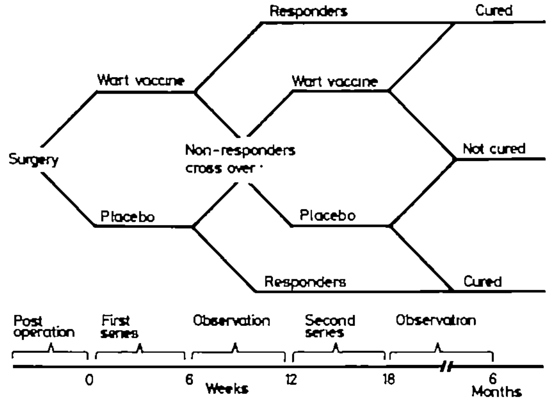


FIG 2 *Schematic representation of study plan*

injections. Those who did not respond, however, were observed for an additional six weeks before crossing over to the second series of vaccinations.

Patients were considered cured if there was no evidence of disease six months after treatment.

STATISTICAL ANALYSIS

The χ^2 test with Yates's correction was used.

Results

The results are summarised in table III. Eighteen patients were treated initially with autogenous wart vaccine and 10 (56%) were cured. Sixteen were initially treated with placebo vaccine and four (25%) were cured ($\chi^2_1 = 2\cdot12$; P $= 0\cdot14$).

TABLE III *Summary of clinical results*

	No of patients		
Results	*Cured (%)*	*Failed*	*Total*
First vaccine			
Wart	10 (56)	8	18
Placebo	4 (25)	12	16
Second vaccine			
Wart	2 (25)	10	12
Placebo	2 (17)	6	8

At cross-over the 12 patients who failed initially to respond to the placebo vaccine were treated with wart vaccine, and two (17%) were cured. Eight patients who failed initially to respond to wart vaccine were treated with placebo and two (25%) were cured. An equivalant P value cannot be directly calculated for the cross-over groups since the patients were no longer randomised because they were preselected by the outcome of the first series of vaccinations. It is apparent, however, that there was even less difference between the treatment results in the cross-over groups.

All patients responded in an all-or-none fashion; there were no partial regressions or incomplete cures.

The mean follow-up period is now 1·3 years with no reported recurrences.

No correlation was observed between the concentration of protein in the vaccines and the patients who were cured.

When the influence of duration of disease is taken into account, the data can be re-examined to compare the wart and placebo vaccines in only those patients with disease for one year or less (table IV). As expected the cure rates for all groups were higher regardless of the form of treatment, but, more importantly, there was much less difference between the two therapies than in the previous analysis, where duration of disease had not been considered ($\chi^2_1 = 0\cdot63$; P $= 0\cdot43$).

TABLE IV *Results in patients with disease for one year or less*

	No of patients		
Results	*Cured (%)*	*Failed*	*Total*
First vaccine			
Wart	10 (77)	3	13
Placebo	4 (50)	4	8
Second vaccine			
Placebo	2 (66)	1	3
Wart	2 (50)	2	4

No virus-type particles were observed in sediment from vaccines which had been negatively stained by phosphotungstic acid and examined by electron microscopy. A few wart specimens from patients with a disease duration of less than a year had intranuclear virus-type particles present.

Discussion

At first glance, a P value of 0·14, although not statistically significant, suggests that wart vaccine may have proved clinically superior to placebo had more subjects been added to the study population. On closer examination of the data, however, little difference was found between the two therapies in the cross-over groups. In addition, there is an unexpected disparity between the initial and cross-over groups treated with wart vaccine (56% against 17% cure rates, respectively), suggesting the possibility of an unanticipated bias which could have skewed the data.

When such a bias was looked for the important influence of the duration of disease was evident. In all patients with disease for one year or less the confirmed cure rate with vaccine or placebo was 86% (18/21) compared with zero (0/13) for those with the disease for more than a year ($\chi^2_1 = 25{\cdot}6$; $P<0{\cdot}001$). Thus, this factor alone is capable of influencing the cure rates of different groups of patients treated by identical methods. It was subsequently discovered that the initial group treated with wart vaccine comprised 72% of patients with disease for one year or less compared with 33% for the cross-over group. Similarly, the overall cure rate in this study was only 56% (18/34) with the mean duration of disease being 3·6 years. This is in contrast to the 94% cure rate reported by Abcarian and Sharon[16] in a large series of patients treated by autogenous wart vaccine where the mean duration of disease was 0·5 years.

Is it possible that cure rates as high as 94% are attributable to either spontaneous remission or placebo effect? When one considers the results of the many studies performed in the 1920s and 1930s on patients with warts using various placebo therapies, this possibility does not seem unlikely. Allington[17] reviewed these works and reported rates of spontaneous remission and placebo cures ranging from 20-89%.

But this is not to say that the immune system does not play a role in the resolution of wart disease. On the contrary, most evidence available today, both serological and histological, indicates that it does. Still, it is not clear what specific factors are capable of influencing the immune system in patients with wart disease or, even less clearly, the mechanisms by which they do so. Autogenous wart vaccine once held promise as one of these specific factors; unfortunately, this theory has not been substantiated by a double-blind controlled study.

In summary, autogenous wart vaccine could not be shown to be more effective for condylomata acuminata than placebo vaccine prepared identically from normal skin. Conversely, it cannot be ruled out that some other antigen common to both normal skin and to condylomata is not responsible for such an action. This possibility would seem unlikely, however, in view of previous studies which have shown comparable cure rates in patients with warts treated solely by injections of saline[18] or distilled water.[19]

Our electron microscope examination of wart tissue showed relatively few cells containing intranuclear papilloma virus-type particles. The vaccines prepared for use in this investigation may thus have had low antigenicity. If it is possible to produce sufficient viral antigen by cell culture or DNA replication methods in the future, then vaccines with sufficient potency to provoke wart involution may result.

This study was supported by a fellowship grant awarded to Dr Malison by the Huntington Institute of Applied Medical Research. Special thanks are offered to Mrs Debbie Collins, Mrs Darlene Leyba, and the nurses of the Pasadena Dispensary for their many hours of voluntary assistance.

References

1. Lee AK, Eisinger M. Cell-mediated immunity (CMI) to human wart virus and wart-associated tissue antigens. *Clin Exp Immunol* 1976; **26:**419-24.
2. Pyrhonen S, Johansson E. Regression of warts, an immunological study. *Lancet* 1975;i:592-6.
3. Boxer RJ, Skinner DG. Condyloma acuminata and squamous cell carcinoma. *Urol* 1977;**9:**72-8.
4. Rhatigan RM, Jiminez S, Chopskie EJ. Condyloma acuminatum and carcinoma of the penis. *South Med J* 1972;**65:**423-8.
5. Orth G, Favre M, Jablonska S, Brylak K, Croissant, O. Viral sequences related to human skin papillomavirus in genital warts. *Nature* 1978;**275:**334-6.
6. Almeida JD, Oriel JD, Stannard LM. Characterization of the virus found in human genital warts. *Microbios* 1969;**3:**225-32.
7. Pyrhonen S. Human wart-virus antibodies in patients with genital and skin warts. *Acta Dermatol* 1978;**58:**427-32.
8. Biberstein H. Versuche uber immunotherapie der Warzen und Kondylome. *Klin Wochenschr* 1925;**4:**638-9.
9. Cormia FE. Autolysate therapy for verruce vulgaris. *Arch Dermatol* 1934;**30:**44-8.
10. Culp OS, Kaplan IW. Condyloma acuminata; two hundred cases treated with podophyllin. *Ann Surg* 1944;**120:**251-6.
11. Powell LC, Pollard M, Jinkins JL. Treatment of condyloma acuminata by autogenous vaccine. *South Med J* 1970;**63:**202-5.
12. Nel WS, Fourie ED. Immunotherapy with 5% topical 5-fluorouracil ointment in the treatment of condyloma acuminata. *S Afr Med J* 1973;**47:**45-9.
13. Ablin RJ, Curtis WW. Condyloma acuminata: treatment by autogenous vaccine. *Ill Med J* 1975;**147:**343-6.
14. Abcarian H, Smith D, Sharon N. The immunotherapy of anal condyloma acuminatum. *Dis Colon Rectum* 1976;**19:**237-44.
15. Vuori J, Alfthan O, Pyrhonen S, Kiistala U, Lassus A. Treatment of condyloma acuminata in male patients. *Eur Urol* 1977;**3:**213-5.
16. Abcarian H, Sharon N. Effectiveness of immunotherapy in the treatment of anal condyloma acuminatum. *J Surg Res* 1977; **22:**231-6.
17. Allington HV. Review of the psychotherapy of warts. *Arch Dermatol Syphilol* 1952;**66:**316-26.
18. Grumach L. Ubel Suffestivbehandlung von Warzen. *Munch Med Wochenschr* 1927;**74:**1093-4.
19. Allington HV. Sulpharsphenamine in the treatment of warts. *Arch Dermatol Syphilol* 1934;**29:**687-90.

Br J Vener Dis 1982;**58:**66

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Lincomycin versus vancomycin in New York City (NYC) medium for the cultural diagnosis of gonorrhoea

Sir,

The following comments are made in response to the letter by A B Hookham (1981;**57:**213).

In 1978 we presented results obtained with varying concentrations of vancomycin and lincomycin incorporated separately in New York City (NYC) medium to determine the degree of their selectivity and sensitivity for isolation of *Neisseria gonorrhoeae* from clinical specimens. Those results indicated that 2 μg/ml vancomycin used in conjunction with colistin, amphotericin B, and trimethoprim lactate in the medium provided adequate selectivity and reduced the percentage of losses of vancomycin-sensitive strains of gonococci observed with 3 μg/ml vancomycin. The same medium with concentrations of 1-4 μg/ml of lincomycin substituted for vancomycin permitted more contamination and yielded fewer recoveries of gonococci compared with 2 μg/ml vancomycin.

Recently (unpublished observations), we again performed similar studies using 3 μg/ml and 4 μg/ml lincomycin in the NYC formulation together with the other three antimicrobial agents mentioned above. In both series of the latter tests our results were in agreement with those noted by Hookham,[2] who used Young's modification B of NYC medium, which contains 1 μg/ml lincomycin.[3]

When NYC medium containing lincomycin was inoculated with anal specimens we found a high percentage of gross contamination (22·6-34%), as a result of which 3·8% of *N gonorrhoeae* strains were lost. By contrast, when anal specimens were streaked on NYC medium containing 2 μg/ml vancomycin, only 5·8% of the plates were moderately or slightly contaminated, with no losses of gonococcal strains. In addition, 1·3-2·9% of *N gonorrhoeae* strains were inhibited on media containing 3 or 4 μg/ml lincomycin. This inhibition of *N gonorrhoeae* strains in media with 3 or 4 μg/ml lincomycin, coupled with poor selectivity, resulted in an overall loss of 5·1% gonococcal strains compared with 1·4% on the medium with 2 μg/ml vancomycin.

At present, therefore, we conclude that vancomycin at 2 μg/ml is the optimal choice for the formulation of NYC medium to be used for any type of specimen. This concentration provides the highest yield of gonococcal isolates by avoiding losses of vancomycin-sensitive strains and by providing sufficient selectivity to prevent losses due to overcontamination. Nevertheless, periodical surveys of the vancomycin sensitivity of prevailing *N gonorrhoeae* strains should be conducted in individual geographical areas, since recently the percentage of vancomycin-sensitive strains has increased in some regions.[4]

Yours faithfully,
Yvonne C Faur
Marion E Wilson

Department of Health,
Bureau of Laboratories,
455 First Avenue,
New York,
NY 10016
USA

References

1. Faur YC, Weisburd MH, Wilson ME. The selectivity of vancomycin and lincomycin in NYC medium for the recovery of *N gonorrhoeae* from clinical specimens. *Health Lab Sci* 1978; **15:**22-7.
2. Hookham AB. Thayer-Martin medium and modified New York City medium for the cultural diagnosis of gonorrhoeae. *Br J Vener Dis* 1981;**57:**213.
3. Young H. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br J Vener Dis* 1978;**54:**36-40.
4. Windall JJ, Hall MM, Washington II JA. Inhibitory effects of vancomycin on *Neisseria gonorrhoeae* in Thayer-Martin medium. *J Infect Dis* 1980; **142:**775.

Obituary

WILLIAM HALDANE DONALD (1922-81)

William Haldane Donald died on 27 June 1981 at the age of 59 at the Derbyshire Royal Infirmary, the hospital he had served with distinction and devotion since 1950.

Educated at Epsom College and the University of Edinburgh, he qualified MB BCh in 1944. His definitive clinical interest became clear in his first appointments as house surgeon and subsequently as clinical tutor in the venereology department of the Royal Infirmary of Edinburgh. He served in the RAMC as a graded specialist in venereology and returned to Edinburgh in 1948. He obtained his Edinburgh MD in 1965 by thesis on the subject of *Trichomonas vaginalis* infection in men.

His appointment to Derby soon extended to Chesterfield, and he worked with H R M Richards, who remained his lifelong friend.

Known affectionately as 'Jock', his special qualities of calm good sense and efficiency earned him the trust and sturdy respect of his colleagues, who expressed this by electing him president of their medical society in 1975 and chairman of the medical committee in 1976. He was also chairman of the Trent RHA advisory subcommittee in venereology from 1975 to 1979.

He supervised the planning of the new department of genitourinary medicine at the Derbyshire Royal Infirmary and saw it built and opened, and the area health authority has approved the naming of this department after him.

His family life was immensely happy and his wonderful practical abilities enabled him to carry out do-it-yourself projects with professional skill. His wife Isobelle, son Iain, and daughters Helen, Sheenagh, and Fiona survive him. Shortly before he died, his heart was gladdened by the news that Fiona had qualified as a doctor. He is greatly missed.—VML.

Br J Vener Dis 1982;**58:**67-70

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea
(Clinical; microbiology; therapy)
Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases
Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (clinical and therapy)

Diagnosis of syphilis
H-J HAGEDORN (University of Dusseldorf, W Germany). *Dtsch Med Wochenschr* 1981;**106:**842.

Syphilis (pathology and experimental)

Cultivation of virulent *Treponema pallidum* in tissue culture
AH FIELDSTEEL, DL COX, AND RA MOECKLI (SRI International, Menlo Park, California, USA). *Infect Immun* 1981; **32:**908-15.

This paper is a milestone in venereological research as it describes clearly with considerable detail the in-vitro culture of *Treponema pallidum*. A cottontail rabbit epithelium cell line was grown in a basal medium (the formula for which is given) and inoculated with three different quantities of *T pallidum* (Nichols strain), 10^6, $2 \cdot 5 \times 10^6$, and 10^7 treponemes. The cell culture was maintained in an environment of 1·5% O_2, 5% CO_2, and 93·5% N_2. After five days the number of organisms had increased to between 8×10^6 and $2 \cdot 59 \times 10^7$, growth continuing over the next 4-7 days to reach a plateau of about 10^8 organisms. The increase in numbers was measured by direct counts of the organisms and by estimating the increase in treponemal DNA; this was greatest when the inoculum was 10^6 organisms. The amount of DNA per treponeme in each of the inoculum groups was consistent ($3 \cdot 14 \pm 0 \cdot 72 \times 10^{-14}$g per treponeme). Virulence was tested after seven days' incubation by inoculating the shaved back of rabbits. In every case a lesion containing treponemes was produced.

Scanning electron microscopy of the cell culture showed the formation of microcolonies on the surface of the epithelial cells.

One important feature of these experiments was that they were repeated in a different laboratory and were shown to be reproducible.

G D Morrison

Helical conformation of *T pallidum* (Nichols strain), *T paraluis-cuniculi*, *T denticola*, *B turicatae*, and unidentified oral spirochetes
DE STEPAN AND RC JOHNSON (University of Minnesota, Minneapolis, USA). *Infect Immun* 1981;**32:**937-40.

The authors used scanning electron microscopy to investigate whether treponemes are flat-wave forms or helices. *T pallidum* was found to exist in both forms, the majority (75%) being a left-handed helix. *T paraluis-cuniculi* existed exclusively as a left-handed helix. *Borrelia turicatae* was found to have a right-handed helix along with *T denticola*. The electron photomicrography is of very good quality in this paper.

G D Morrison

Gonorrhoea (clinical)

Diagnosis of gonorrhoea
H-J HEITE (Waldkirch, W Germany). *Dtsch Med Wochenschr* 1981;**106:**872.

Ocular gonococcal infection with little or no inflammatory response
JK PODGORE AND KK HOLMES (Madigan Army Medical Centre, Tacoma, Washington, USA). *JAMA* 1981;**246:**242.

Gonorrhoea (microbiology)

Molecular and phenotypic characterization of penicillinase-producing *Neisseria gonorrhoeae* from Canadian sources
JAR DILLON, P DUCK, AND DY THOMAS (Toronto, Canada). *Antimicrob Agents Chemother* 1981;**19:**952-7.

Pathogenic mechanisms of *Neisseria gonorrhoeae:* observations on damage to human fallopian tubes in organ culture by gonococci of colony type 1 or type 4
Z McGEE, AP JOHNSON, AND D TAYLOR-ROBINSON (Vanderbilt University School of Medicine, Nashville, Tennessee, USA). *J Infect Dis* 1981;**143:**413-22.

Studies of toxicity of *N gonorrhoeae* for human fallopian tube mucosa
MA MELLY, CR GREGG, AND ZA McGEE (Vanderbilt University School of Medicine, Nashville, Tennessee, USA). *J Infect Dis* 1981;**143:**423-31.

Toxic activity of purified lipopolysaccharide of *N gonorrhoeae* for human fallopian tube mucosa
CR GREGG, MA MELLY, GG HELLERQVIST, *et al* (Vanderbilt University School of Medicine, Nashville, Tennessee, USA). *J Infect Dis* 1981;**143:**432-9.

Monoclonal antibodies against *Neisseria gonorrhoeae:* production of antibodies directed against a strain-specific cell-surface antigen

I NACHAMKIN, JG CANNON, AND RS MITTLER (Department of Clinical Pathology, Virginia University, Richmond, Virginia, USA). *Infect Immun* 1981;**32:**641-8.

Identification and isolation of novel pilus types produced by variants of *N gonorrhoeae* P9 following selection in vivo

PR LAMBDEN, JE HECKELS, H McBRIDE, AND PJ WATT (University of Southampton, Southampton, UK). *FEMS Microbiol Letters* 1981;**10:**339-42.

Antigen-specific serotyping of *N gonorrhoeae:* characterisation based upon principal outer membrane protein

TM BUCHANAN AND JF HILDEBRANDT (United States Public Health Service Hospital, Seattle, Washington, USA). *Infect Immun* 1981;**32:**985-94.

Typing of gonococci has been attempted by many different means but none has proved sufficiently simple to find universal application. The system described is of considerable interest because it explores the distribution of an important constituent of the outer membrane of the gonococcus and, once reagents have been produced, allows commendably quick typing of gonococcal isolates.

An ELISA technique was used to detect differences in the principal outer membrane protein (protein 1) of the gonococcus. It involved extraction and purification of the protein by cell disruption and ultracentrifugation with or without chromatography using Sepharose 6B (which has an exclusion limit of 4×10^6 molecular weight). Fractions contaminated with less than 4% lipopolysaccharide were coated on to polystyrene tubes and used in an ELISA test both directly to detect antibody and indirectly to measure and identify protein 1 on the surface of gonococci in inhibition assays.

No differences in concentration of protein 1 were detected in different Kellogg colonial types or in opacity variants of the same strain.

Nine serotypes of protein 1 were identified (types 1-9). An initial table indicates that only types 1 and 2 showed any significant antigenic sharing (about 30%), the other types showing little cross-reaction.

When the system was used to type 125 strains of gonococci, 124 could be typed. Of these, 35% typed with a single protein 1 serotype; the others reacted with more than one. The reasons for this multiple reactivity are discussed at length but the system as it stood showed considerable selectivity; there appeared to be a correlation between the clinical syndrome produced by a gonococcus and its protein 1 type.

A E Jephcott

Isolation and characterisation of a *β*-lactamase-specifying plasmid in a strain of *N gonorrhoeae*

JC LEFEVRE, MF PRERE, AND F BOUVIER (Faculty of Medicine, Toulouse University, Toulouse, France). *Ann Microbiol* 1981;**A132:**283-92.

Effects of proteolytic enzymes on the outer membrane proteins of *N gonorrhoeae*

MS BLAKE, EC GOTSCHLICH, AND J SWANSON (Rockefeller University, New York, USA). *Infect Immun* 1981;**33:**212-22.

Gonorrhoea (therapy)

Treatment of gonorrhoea

H-J HEITE (Waldkirch, West Germany). *Dtsch Med Wochenschr* 1981;**106:**873.

Non-specific genital infection

Microtest procedure for isolation of *Chlamydia trachomatis*

BL YODER, WE STAMM, CM KOESTER, AND ER ALEXANDER (University of Washington, Seattle, USA). *J Clin Microbiol* 1981;**13:**1036-9.

Mycoplasmas in male genital tract infections

T KUMAR, B KUMAR, PJ ASNANI, *et al* (Punjab University, Chandigarh, India). *Indian J Med Res* 1981;**73:**715-9.

Prevalence of *Chlamydia trachomatis* and *N gonorrhoeae* in two different populations of women

WR BOWIE, CJ BORRIE-HULME, LM MANZON, *et al* (University of British Columbia, Vancouver, BC, Canada). *Can Med Assoc J* 1981;**124:**1477.

Chlamydia trachomatis infection in mothers and infants—a prospective study

AD HEGGIE, GG LUMICAE, LA STUART, AND MT GYVES (University Hospital, Cleveland, Ohio, USA). *Am J Dis Child* 1981;**135:**507-11.

Diagnosis of infectious non-gonorrhoic urethritis

W BREDT (University of Freiburg, W Germany). *Dtsch Med Wochenschr* 1981;**106:**909-10.

A newly discovered mycoplasma in the human urogenital tract

JG TULLY, D TAYLOR-ROBINSON, RM COLE, AND DL ROSE (National Institute of Health, Bethesda, Maryland, USA). *Lancet* 1981;i:1288-91.

A new mycoplasma, serologically distinct from all other known mycoplasmas, was isolated from urethral specimens from two of 13 men with non-gonococcal urethritis. Repeatable isolation and propagation was accomplished by use of a special culture medium. The organisms adhered to glass or plastic, erythrocytes, and monkey kidney cells. This property appears to be associated with surface material restricted to the area of a terminal structure of the flask-shaped mycoplasmas. Although data are insufficient to implicate the new mycoplasmas in human disease, the fact that they are unique, extremely fastidious, and have adherence properties has stimulated efforts to assess their pathogenicity and possible role in human urogenital disease.

Authors' summary

Polypeptide composition of *Chlamydia trachomatis*

SH SALARI AND ME WARD (University of Southampton, Southampton, UK). *J Gen Microbiol* 1981;**123:**197-209.

Experimental vaccination of monkeys and man with *Chlamydia trachomatis* suggests that immunity to ocular infection is type specific. Unfortunately, evidence also suggests that vaccinated subjects who subsequently become infected with chlamydia of heterologous serotype develop more severe ocular disease than unvaccinated controls, presumably as a result of hypersensitivity reactions to chlamydial group-specific antigens. Characterisation of chlamydial antigens is thus essential to an understanding of the pathogenesis of chlamydial ocular and genital tract infections and the development of a vaccine. Unfortunately, the chlamydia responsible for trachoma in the third world and for genital tract infections in developed countries are difficult to grow and purify in adequate quantity for immunochemical analysis. The methods used to grow and purify *C trachomatis* strains for such studies and the polypeptide composition of strains representative of 14 of the 15 chlamydial serotypes are described in this paper.

It has been known for some time that the susceptibility of HeLa 229 cells to infection with most *C trachomatis* serotypes is increased if the cells are first washed in DEAE-dextran before chlamydial challenge. In this study the optimal concentration of DEAE-dextran required to maximise chlamydial yields was determined for each serotype. After homogenisation to release the chlamydia from within the infected HeLa cells the former were separated from host-cell material by centrifugation on a metrizoate density gradient, exploiting the fact that chlamydia and host-cell material have different buoyant density in aqueous media. The purity of the separated chlamydia was rigorously confirmed by several different criteria. The purified chlamydia were solubilised in detergent and the liberated polypeptides characterised by electrophoresis in the presence of detergent on a molecular-size-limiting gradient of polyacrylamide. Not surprisingly, chlamydia possessed a large number of unique polypeptides, many of which were present in all 14 serotypes examined. The most dominant polypeptide, however, which the authors believe represents the major chlamydial surface protein, varied in its molecular weight from 38 000-42 000 daltons according to serotype. Interestingly, chlamydial serotypes responsible for either trachoma, non-specific genital tract infection (NSGI), or lymphogranuloma venereum (LGV) each had their own characteristic major polypeptide. Thus, it was postulated that the major chlamydial polypeptide might be of evolutionary or pathological importance. In addition, LGV agents possessed a unique polypeptide of 118 000 daltons not found among the strains of chlamydia causing trachoma or uncomplicated genital infection (NSGI).

The chlamydial surface must be an important target for host immune defences as well as being vital in the attachment and penetration of chlamydia into host cells. Using a technique which selectively labels only proteins present at the microbial surface it was suggested that the major chlamydial polypeptide and polypeptides of 29 000 and 155 000 daltons (molecular weight) were located at the chlamydial surface. Comparison with other work suggests the chlamydial 29 000-dalton polypeptide is type specific (possibly responsible for type-specific immunity) and the 155 000-dalton polypeptide is species specific.

M E Ward

Studies on *Chlamydia trachomatis* as a cause of lower urogenital tract infection

G JOHANNISSON (University of Göteborg, Göteborg, Sweden). *Acta Dermato-venereol* 1981;**93** suppl.

This study (which is based on other publications by the author and his colleagues) examines the local prevalence, natural history, infectivity, and susceptibility to antibiotics in vitro and in vivo of infections of the lower genital tract with *C trachomatis.*

In men with non-gonococcal urethritis attending the venereal diseases clinic in Göteborg, 43% and 44% (two studies) were infected with *C trachomatis* as were 73% of men with post-gonococcal urethritis. Five per cent of men without urethritis were also isolation-positive, as were 38% and 27% of women in two further studies. Fifteen per cent of women were found to be infected in the urethra alone. The prevalence in men with NGU attending a urology clinic was 26%, of whom 26/96 also had chlamydia in their prostatic fluid. The incubation period of chlamydial urethritis was one week or less in 43% of men, but women were often asymptomatic. Infections in both sexes were found to be self-limiting in a small series of patients left untreated, and the infectivity of *C trachomatis* was less than that of *Neisseria gonorrhoeae.*

In-vitro studies with 13 antibiotics to four strains of *C trachomatis* showed that erythromycin, pivampicillin, oxytetracycline, and doxycycline were the most effective; the latter was considered to be the best treatment for NGU in men when given for one week, although it was thought that more prolonged therapy should be given to women.

The supplement includes an introduction into the history, classification, isolation, and serology of *C trachomatis;* details of specimen collection and laboratory methods are clearly defined. A general discussion provides a concise summary of the many aspects of chlamydial infections and, with 145 references, this publication will be of value to all those with an interest in this field.

J R Willcox

Non-gonococcal urethritis—epidemiological and etiological study in Italy

R CEVENINI, C VAROTTI, F RUMPIANESI, *et al* (Ospedale S Orsola, Institute of Microbiology, Bologna, Italy). *Boll Inst Sieroter (Milan)* 1980;**59**:599-604.

One-week treatment of chlamydia-positive urethritis with doxycycline and tetracycline chloride in males

T JUVAKOSKI, J LAUHARANTA, L KANERVA, AND A LASSUS (Department of Dermatology, University of Helsinki, Finland). *Acta Dermatovenereol* 1981;**61**:273.

Effect of cycloheximide in the infective yield of a genital strain of *Chlamydia trachomatis* in McCoy cells

P KARAYIANNIS, D HOBSON, AND N LEE (Department of Medical Microbiology, University of Liverpool, Liverpool, UK). *Infect Immun* 1981;**33**:309-31.

Reiter's disease

Reiter's syndrome—evaluation of preliminary criteria for definite disease

RF WILLKENS, FC ARNETT, T BITTER, *et al* (University of Washington, Seattle, USA). *Arthritis Rheum* 1981;**24**:844-9.

A retrospective attempt to define 'definite' Reiter's syndrome (RS) was made by evaluating 66 clinical features in 83 patients with typical RS and comparing them with a control group (53 with ankylosing spondylitis, 33 with seronegative rheumatoid arthritis, 53 with psoriatic arthritis, and 27 with gonococcal arthritis).

From the data collected the proposal was made that 'Reiter's syndrome consists of an episode of peripheral arthritis of more than one month's duration occurring in association with urethritis and/or cervicitis'. The percentage of cases of RS satisfying these criteria (that is, sensitivity) was 84·3% at the initial episode and 97·6% when subsequent attacks were included. Specificity (percentage of control patients not satisfying the criteria) varied from 96·2% to 100%.

The authors compare the value of their proposals with those used in the diagnosis of rheumatoid arthritis and systemic lupus erythematosus, emphasising the inherent difficulties in RS due to the transient nature of the disease in some and the problem of long-term follow up.

Unfortunately, the definition of urethritis in this study is vague and there are no diagnostic criteria given for 'cervicitis,' although seven of 83 patients with RS were female.

R S Pattman

Trichomoniasis

A double-blind study of the value of treatment with a single dose of tinidazole of partners to females with trichomoniasis

J LYNG AND J CHRISTENSEN (Copenhagen, Denmark). *Acta Obstet Gynecol Scand* 1981;**60**:199-202.

Candidosis

Prevalence of yeasts and fungi other than *Candida albicans* in the vagina of normal young women

MJ GOLDACRE, LJR MILNE, B WATT, N LOUDON, AND MP VESSEY (Universities of Oxford and Edinburgh, UK). *Br J Obstet Gynaecol* 1981;**88**:596-600.

Genital herpes

Regular review: recurrent herpes simplex: the outlook for systemic antiviral agents

HJ FIELD AND D WILDY (University of Cambridge, UK). *Br Med J* 1981;**282**: 1821-22.

Growth of herpes simplex virus types 1 and 2 in tissues of fertile hens eggs in ovo and in vitro

FD RODGERS (Public Health Laboratory, University of Nottingham, UK). *Br J Exp Pathol* 1981;**62**:317-22.

Herpesvirus-induced antigens in squamous-cell carcinoma in situ of the vulva

RH KAUFMAN, GR DREESMAN, J BUREK, OM KORHONEN, DO MATSON, *et al* (Baylor College of Medicine, Houston, Texas, USA). *N Engl J Med* 1981;**305**:483-8.

Squamous cell carcinoma in situ of the vulva appears to be increasing, even in women under 40 years. An HSV-2-specific DNA-binding protein was detected in biopsy specimens from nine out of 10 patients with this condition. In one of these patients there was severe dysplasia, and in another vulval carcinoma in situ.

In the controls, who included patients with condylomata acuminata and those attending for routine examinations, the findings were negative. In one patient the vulval changes developed six weeks after a primary HSV-2 infection of the vulva and in another after many years.

The results of this series must be considered speculative, but they should alert the physician to the possibility that carcinoma of the female genital tract may be induced by HSV-2 infection. Further large-scale investigations are needed.

G W Csonka

Other sexually transmitted diseases

Hepatitis A in homosexuals

A MINDEL AND R TEDDER (Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, London, UK). *Br Med J* 1981;**282**:1666.

Miscellaneous

Prevalence of bacteria in the vagina of normal young women

B WATT, MJ GOLDACRE, N LOUDON, DJ ANNAT, RI HARRIS, AND MP VESSEY (University of Edinburgh and Oxford, UK). *Br J Obstet Gynaecol* 1981;**88**: 588-95.

In a study of factors related to the prevalence of bacteria in the vagina of 1498 women attending a family planning clinic, anaerobes were significantly commoner in women with an abnormal vaginal discharge on clinical examination, in those with a history of a troublesome vaginal discharge, and in those who used an intrauterine contraceptive device (IUCD). The association between anaerobes and abnormalities of vaginal discharge was found both in women who used the IUCD and in women who did not.

No significant associations between symptoms and other bacterial species or combinations of species were evident. Coliforms were significantly commoner in women who did not use tampons, in those who used the contraceptive diaphragm, and in those who had received recent antibacterial or antifungal treatment. Lactobacilli were significantly commoner in women who used the contraceptive pill and significantly less common in those who harboured *Candida albicans*, anaerobes, coliforms, or enterococci and in those who had been treated with antibacterial drugs. No important associations were found between the presence of bacteria and the patient's age, parity, or social class.

Authors' summary

Association of Behcet's disease with HLA-B5 in the Mexican Mestizo population

C LAVALLE, ALARCON-SEGOVIA, DEL GUIDICE-KNIPPING, AND FRAGA (Mexico City, Mexico). *J Rheumatol* 1981;**8**:325-8.

Treatment of *Haemophilus vaginalis* vaginitis

M MALOUF, M FORTIER, G MORIN, AND J-L DUBE (Quebec, Canada). *Obstet Gynecol* 1981;**57**:711-4.

Single 2 capsule dose: 92·5% success in gonorrhoea

Eradacin

acrosoxacin
(rosoxacin I.N.N.)

Single oral dose of only 2 capsules for convenience, acceptance and compliance.

92.5% clinical success reported (n=797).[1]
In apparent clinical failures, subsequent *in vitro* testing has shown 100% sensitivity of all strains.[1]

Active against all strains of gonococcus tested.[2,3,4]

Rapid bactericidal activity comparable to that of penicillin.[1,2] Symptomatic improvement in 2 days and bacteriological cure after 3 days observed.[1]

No interference with syphilis serology reported.

Each capsule contains 150mg acrosoxacin. Supplied in bottles of 20 capsules. Basic N.H.S. cost of one treatment (2 capsules) £2.22. PL0071/0168. **Dosage** Adults should be given a single dose of two capsules (300mg) preferably on an empty stomach. **Precautions** Safe use during human pregnancy has not yet been established. Use with caution in the presence of renal or hepatic impairment. Eradacin can produce lesions in weight-bearing joints in young animals receiving high, single or repeated doses. The relevance of this in man is not known. Headaches and gastro-intestinal upsets may occur. Dizziness and drowsiness may be experienced; patients should therefore be warned not to operate machinery or drive if affected. Eradacin is a trade mark. Further information is available from

SRL
Sterling Research Laboratories,
St. Mark's Hill,
Surbiton-upon-Thames, Surrey.

1. Data on file: Sterling Research Laboratories.
2. Braveney, I. & Machka, K. 1980. Arzneim-Forsch./Drug Res. 30(II): 1476-1478.
3. Seth, A. D. 1981. J. Antimicrob. Chemother. 7: 331-334.
4. Warren, C. A. et al. 1981. Brit. J. Vener. Dis. 57: 33-35.

M.S.S.V.D.

The Medical Society for the Study of Venereal Diseases

11 Chandos Street, Cavendish Square, London W1M 0EB
President: Dr. Jack Barrow

The object of the Society is to bring together those interested or engaged in the diagnosis, treatment, and public health aspects of venereal diseases. The Society meets regularly in London for the reading of papers, discussions, and presentation of cases. An out-of-London meeting is held in the Spring.

Annual subscription for membership of the Society is £20 for all members.

Applications for membership are very welcome and forms can be obtained from the Honorary Secretary, Dr. M. A. Waugh, Department of Genito-Urinary Medicine, The General Infirmary Great George Street, Leeds LS1 3EX.

Proposed Programme 1982

Friday 26th March 1982	Herpes genitalis — general reveiw (including experience with Acyclovir) Assoc. Prof. Lawrence Corey
Friday 30th April 1982	Candidiasis — Immunology Prof. J. R. Hobbs — Mycosis with Special Reference to Genital Candidiasis Prof. D. W. Mackenzie
Friday 21st-23rd May 1982	Spring Meeting in Jersey

Venereology books from Blackwell

Lecture Notes on Sexually Transmitted Diseases

R. Nicol Thin MD, FRCPE
Spring 1982. 260 pages, 65 illustrations.
Paper, £7.25

The common sexually transmitted diseases and allied disorders are clearly and simply described in this text. Much of the material is presented in tabular and list form in order to assist easy assimilation and to emphasise important points. While primarily intended for undergraduate medical students, the book will also be of value to postgraduates, nurses and other groups such as contact tracers.

Contents: Epidemiology and control of sexually transmitted diseases; Clinical examination; Early acquired syphilis; Antibody tests; Late acquired syphilis; Congenital syphilis; Gonorrhoea; Management of gonorrhoea; Non-specific genital infection; Reiter's Disease; Trichomoniasis; Candidiasis; Sexually transmitted viral conditions; Parasitic infestations; Sexually transmitted diseases more commonly seen in hot climates; Other genital conditions; Non-venereal treponemal conditions; Diseases found in homosexuals.

Venereological Medicine

Pocket Consultant series

GRANT McINTYRE
MEDICAL & SCIENTIFIC

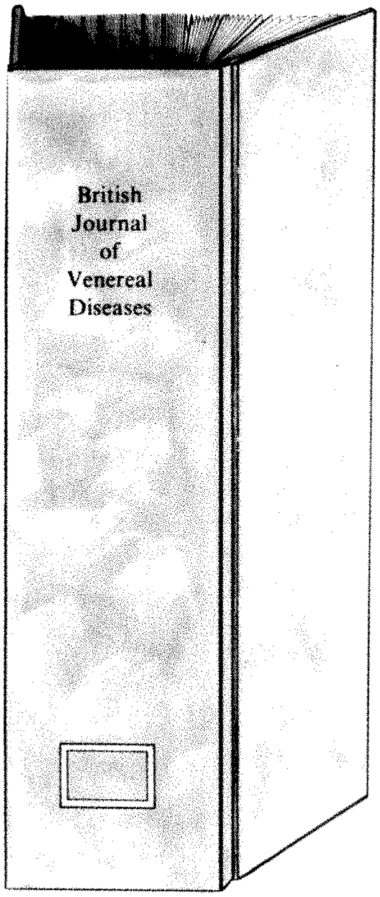
R.R. Willcox MD, FRCP and J.R. Willcox MBBS, MRCS, LRCP, DCH
1982. 352 pages, 80 illustrations. Paper, £9.95

This practical vade mecum covering all aspects of the subject will prove an indispensable reference for medical students, senior nurses specializing in the subject, laboratory staff, social health workers, junior doctors and those studying for the Diploma of Venereology or equivalent examinations.

Contents: Introduction; Standard management of patients; Conditions associated with genital discharges; Gonorrhoea; Non-specific genital infection; Candidiasis; Trichomoniasis; Spirochaetes and treponemes, Treponematoses; Syphilis; Chancroid; Lymphogranuloma venereum; Scabies; Pubic lice; Genital warts; Molluscum contagiosum; Herpes; Infective hepatis; Other conditions; Reactions to therapy; Management techniques; Drug names table.

Blackwell Scientific Publications

Available January 1982



'CORDEX'

SELF-BINDING CASES
for easy and instant reference

Made to hold two volumes (12 issues) of your **BRITISH JOURNAL OF VENEREAL DISEASES.** Each case is handsomely bound with a colour to complement the journal. Spring nylon cords, to carry the journals, slide freely along the spine of the binder. The name of the journal is gold-blocked on the spine. Date labels are also provided.

Price: Inland £4.00; Overseas US$12.00
including postage (air mail overseas) and packing

ORDER NOW

From: The Subscription Manager,
BRITISH JOURNAL OF VENEREAL DISEASES
BMA House, Tavistock Square, London WC1H 9JR

Reduced Subscription Rates for Junior Hospital Doctors Residents and Interns...

British Journal of Venereal Diseases

is available to junior hospital doctors in all parts of the world for a maximum of four consecutive years at REDUCED Annual Subscription Rates. (Thereafter, or when reaching consultant status this special offer will no longer apply.) The following reduced rates apply for the six issues published in 1982.

Inland £20.00

Overseas US$46.00

(The full rates are: Inland £31.00; Overseas US$76.00)

The opportunity to subscribe to this journal of international authority and repute at a substantially reduced subscription rate is one not to be missed by junior doctors seeking to specialise in venereology.

Please complete the adjacent order form and send for your subscription NOW.

Further order forms can be supplied on request.

ORDER FORM

BMA Publications
BMA House, Tavistock Square
London WC1H 9JR, England

Please enter my subscription to

BRITISH JOURNAL OF VENEREAL DISEASES

☐ United Kingdom and Irish Republic £20.00
☐ Overseas US$46.00
☐ My remittance for .... is enclosed
(Please tick appropriate boxes)

NAME ....

....

ADDRESS ....

....

....

To be completed by Consultant

I hereby confirm that the above mentioned doctor is a member of the junior hospital staff/resident/intern at ....

....

Signed ....

BRITISH JOURNAL OF VENEREAL DISEASES

Sexually Transmitted Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $25.00 outside the United States ($22.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $33.00 and $36.00 in the United States and $37.00 and $40.00 in all other countries.

William O. Harrison, M.D.
American Venereal Disease Association
P.O. Box 22349
San Diego, California 92122 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $25.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases*.

☐ I wish to enter a Non-Member or institutional subscription ($33.00 and $36.00 U.S., all other countries $37.00 and $40.00).
(Mail non-member or institutional subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105).

Name ______________________

____

Address ______________________

City ______________ State Country ______________ Zip ______________

British Journal of Venereal Diseases February 1982 Vol 58 No 1

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Morphological destruction of cultured cells by the attachment of *Treponema pallidum*
T J FITZGERALD, L A REPESH, AND S G OAKES *page* 1

Demonstration of specific 19S(IgM) antibodies in untreated and treated syphilis: comparative studies of the 19S(IgM)-FTA test, the 19S(IgM)-TPHA test, and the solid-phase haemadsorption assay
F MÜLLER AND E-G LINDENSCHMIDT *page* 12

Alterations in T lymphocytes and T-lymphocyte subpopulations in patients with syphilis
JØRGEN R JENSEN AND ELLIS FROM *page* 18

In-vitro activity of 21 antimicrobial agents against *Neisseria gonorrhoeae* in Brussels
B GORDTS, R VANHOOF, J M HUBRECHTS, R DIERICKX, H COIGNAU, AND J P BUTZLER *page* 23

Penicillinase-producing gonococcal strains in Zambia: observations on treatment failures
ATTILI V RATNAM, MOHMEDALI I PATEL, RAPHAEL C MULENGA, AND SUBHASH K HIRA *page* 29

Aetiology and management of non-specific vaginitis
I M DATTANI, A GERKEN, AND B A EVANS *page* 32

Quantitative study of *Chlamydia trachomatis* in genital infection
H MALLINSON, O P ARYA, AND A D GODDARD *page* 36

Does psychiatric illness affect the recurrence rate of genital herpes?
DAVID GOLDMEIER AND ANTHONY JOHNSON *page* 40

Incidence of HSV-1 and HSV-2 isolated from a group of patients with herpes genitalis in Sheffield
I G BARTON, G R KINGHORN, S NAJEM, L S AL-OMAR, AND C W POTTER *page* 44

Confirmation of herpes simplex virus type 2 infections in herpes-like genital lesions by a simple complement-fixation test
MINAS ARSENAKIS AND JOHN T MAY *page* 48

Aseptic meningitis and herpes simplex proctitis: a case report
W A ATIA, C S RATNATUNGA, C GREENFIELD, AND S DAWSON *page* 52

Thiamphenicol in the treatment of chancroid in men
A S LATIF *page* 54

International symposium on trichomoniasis: the Polish medical scene
R D CATTERALL *page* 56

Cell-mediated immunity in HBeAg-positive homosexuals with chronic liver disease
L VIOLA, I G BARRISON, F PARADINAS, J C COLEMAN, AND IAIN M MURRAY-LYON *page* 59

Autogenous vaccine therapy for condyloma acuminatum: a double-blind controlled study
MICHAEL D MALISON, RODNEY MORRIS, AND LAWRENCE W JONES *page* 62

Correspondence *page* 66

Obituary *page* 66

Abstracts *page* 67

ASTM CODEN: BJVDAK 58 (1) 1-70 (1982)

ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

British Journal of
Venereal Diseases

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

A E WILKINSON
(Editor)
B A EVANS
(Assistant Editor)
A McMILLAN
(Assistant Editor)
G D MORRISON
(Assistant Editor, Abstracts)

J BARROW
(President, MSSVD)
M WAUGH
(Hon Sec, MSSVD)
M W ADLER
G CAUSSE
G CSONKA

L M DRUSIN
J M KNOX
I PHILLIPS
E STOLZ
R N THIN
J WALLIN

EDITOR,
British Medical Journal

P D SIMMONS
(Indexer)
SHUNA JUDGE
(Technical Editor)

This *Journal,* founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A McMillan, Dept of Genitourinary Medicine, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals,* available from the Publishing Manager, *British Medical Journal,* BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; **55**: 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS, Rodin P. *Venereal Diseases.* 4th ed. London: Baillière and Tindall, 1980.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 214-22.

PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £31.00 in the United Kingdom and Republic of Ireland and US$76.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US$76.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal,* 1172 Commonwealth Avenue, Boston, Mass. 02134. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases,* BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1982 by the *British Journal of Venereal Diseases.* This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.

16.9.8

Prescribing information

Dosage

Treatment; adults and children over 12 years; 100ml by intravenous infusion eight-hourly administered 5ml per minute. Oral medication with 400mg three times daily should be substituted as soon as this becomes feasible. Treatment for seven days should be satisfactory in most cases. Children under 12 years; the intravenous dose is based on 1.5ml (7.5mg metronidazole) per kg bodyweight and the oral dose on 7.5mg per kg bodyweight.

Prevention

Adults and children over 12 years: 100ml by intravenous infusion immediately before, during or after operation, followed by the same dose eight-hourly until oral medication (200 to 400mg three times daily) can be given to complete a seven-day course. Children under 12 years; the intravenous dose is based on 1.5ml (7.5mg metronidazole) per kg bodyweight and the oral dose on 3.7 to 7.5mg per kg bodyweight.

Contra-indications

There are no absolute contra-indications to the use of Flagyl.

Precautions

Regular clinical and biological surveillance advised if duration of treatment exceeding ten days considered necessary; may enhance the effect of oral anti-coagulants. Avoid alcohol, use in pregnancy, lactation. Side-effects; drowsiness, dizziness, headache, ataxia, skin rashes, pruritus, incoordination of movement and darkening of urine (due to metabolite) have been reported but very rarely. A few instances of peripheral neuropathy have been reported during intensive and/or long term treatment. A moderate leucopenia has been reported but the white cell count has always returned to normal before or after treatment has been completed.

Presentation/Cost

(Jan '82) 21x200mg tablets (PL 0012/5256) £2.12. 10x400mg tablets (PL 0012/0084) £1.92. Boxes of 10x100ml bottles, injection solution 0.5% w/v (PL 0012/0107). Direct Hospital Price £4.12 per 100ml. Boxes of 48x100ml injection 0.5% w/v in Viaflex* containers (PL 0012/0107) Direct Hospital Price £4.12 per 100ml. Flagyl-S Suspension (PL 0012/0131) 200mg metronidazole equivalent in each 5ml, £4.75 per 125ml bottle.

Wherever you operate

There's a Flagyl presentation to protect against anaerobic infection in every patient and in every procedure.

- **The 100ml injection bottle, now with the easily-opened ring-pull top**
- **The new, lightweight Viaflex* infusion mini-bag**
- **Two strengths of tablets and suppositories for flexibility of dosage**
- **Flagyl-S Suspension for all those unable or unwilling to swallow tablets**

FLAGYL and FLAGYL-S

metronidazole **benzoylmetronidazole Suspension**

anaerobicides for all regions

M&B May & Baker

Flagyl is a trade mark of May & Baker Ltd., Dagenham, Essex RM10 7XS.
*Registered trade mark of Baxter Travenol Laboratories

cribing Information: Presentations *Vaginal Tablets* Canesten is available either as 200mg vaginal tablets in packs of three with applicator or as 100mg vaginal tablets in packs of six with
licator. *Vaginal Cream* 2.0% clotrimazole cream in tubes of 35g with six applicators. *Canesten Duopak* Six 100mg vaginal tablets with applicator plus 20g cream containing 1.0% clotrimazole.
age *Vaginal Tablets 100mg* Two vaginal tablets should be inserted at night for three consecutive days. Alternatively, one vaginal tablet daily for six days. *Vaginal Tablets 200mg* One vaginal
t should be inserted at night for three consecutive days. *Vaginal Cream* One filled applicator (5g) should be inserted twice daily for three consecutive days or once daily for six consecutive
preferably at night. *Duopak* Vaginal Tablets 100mg as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to
ent re-infection. **Side effects** Rarely patients may experience transient local mild burning or irritation. **Basic NHS Costs** 6 x 100mg vaginal tablets with applicator £2.13; 3 x 200mg vaginal
ts with applicator £1.99; Cream 35g with applicators £4.16; Duopak £3.47. **Product Licence Numbers** *Vaginal Tablets 100mg*, PL0010/0015. *Vaginal Tablets 200mg*, PL0010/0072, *Vaginal*
m, PL0010/0077. *Duopak*, PL0010/0016 and PL0010/0015.

Further information is available fr
Bayer UK Limited
Pharmaceutical Div
Haywards Heath
West Sussex RH16
Telephone: Haywa
Heath (0444) 4141

®Registered trade mark of Bayer, (

Br J Vener Dis 1982;**58**:71

Editorial

Statistical significance: use and misuse

The use of statistical methods in the evaluation of the results of a scientific investigation is usually indispensable. A wide variety of tests of statistical significance, each with its own inherent advantages and disadvantages, are available. The choice of the most appropriate method is sometimes difficult and is often a matter of experience. Through the use of incorrect tests, misinterpretation of the results of a scientific study can occur and undermine the value of otherwise useful data. Although several short textbooks on medical statistics are readily available,[1 2] authors are strongly encouraged to seek the advice of a professional statistician on the methods used in their studies before submission of papers.

Even cursory examination of published papers in this and other journals shows that the term 'statistically significant' is variably interpreted. For example, in volume 56 (1980) of the *British Journal of Venereal Diseases* one group of authors claimed significance when the probability of the event occurring by chance was 1 in 8·3. In the same volume another group of workers used the term 'significant' when there was a chance probability of about 1 in 5000. To avoid possible confusion it is suggested that contributors to the *Journal* should not use the term 'significant' of $P>0\cdot05$.

Authors should ensure that the statistical methods used in their studies are clearly indicated. When used, values for χ^2 should be given in the appropriate section of the paper. If the data presented are insufficient for an independent statistical assessment of the results of an investigation (for example, when Student's *t* test is used without quoting mean and standard deviation), the authors should intimate from whom such information may be obtained.

A McMILLAN

References

1. Bradford Hill A. *Principles of Medical Statistics*, 9th ed. London: The *Lancet*, 1971.
2. Swinscow TDV. *Statistics at Square One*. London: BMA, 1976.

Br J Vener Dis 1982;**58:**72-85

IV Harrison Lecture 1981: the international venereological scene as viewed by Harrison and St Mary's*

R R WILLCOX

From St Mary's Hospital, London

Introduction

Venereology has always had its international aspects extending back through the time when each country in Europe blamed another for the introduction of syphilis at the end of the fifteenth century to as far as Biblical times.[1] Notable early legislation with international relevance affecting the venereal diseases was the Copenhagen regulations of 1802, which provided for the free treatment of foreign seamen,[2] but the starting point for multinational agreement and cooperation in matters of health was the Geneva convention of 1864, which regulated the management of the wounded in war.

Founding of the London Lock Hospital

This was originally founded by William Bromfield (fig 1), a surgeon at St George's Hospital, near Hyde Park Corner, in 1746 (fig 2).[3 4] It was concerned not only with the treatment of venereal diseases but also with the moral welfare and rehabilitation of patients. The Lock Hospital was moved to the Harrow Road in 1842 to a site purchased from the executors of the late Sarah Siddons, the actress; its outpatient department was opened a few years later in Dean Street. The hospital's imposing chapel was consecrated in 1847 with a resident chaplain, the Reverend T Garnier, later Dean of Lincoln,[5] who was also appointed to the immediately adjacent Paddington workhouse, which opened in the same year.[6] The workhouse was later to be integrated into an expanded Paddington Infirmary, now St Mary's Hospital. The site of the present postgraduate centre is where the chapel once stood.

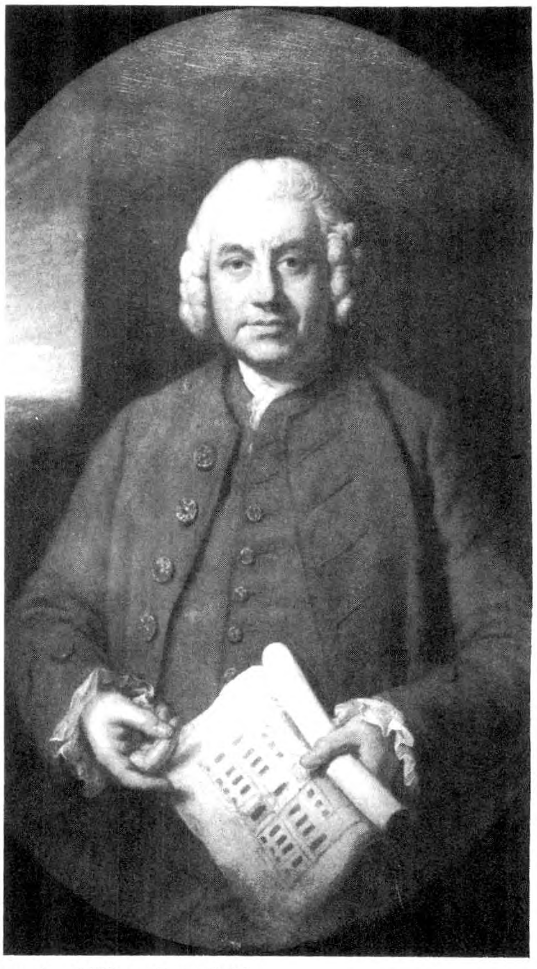


FIG 1 *William Bromfield*

Founding of St Mary's Hospital

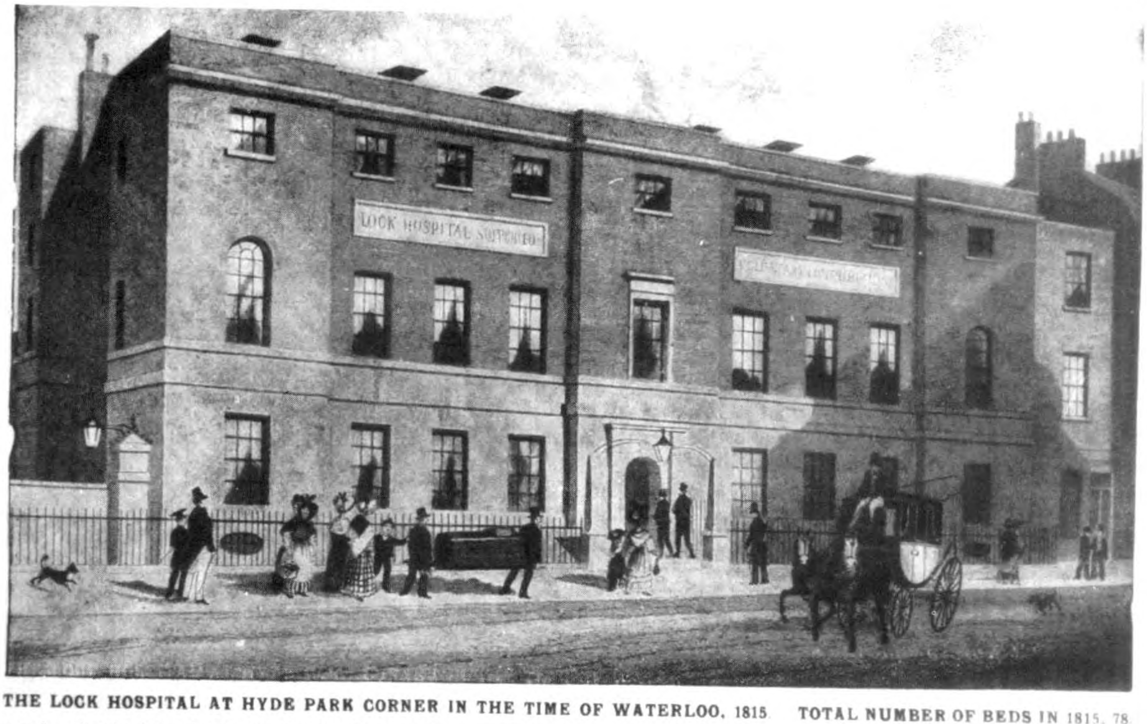
St Mary's Hospital in Praed Street opened in 1851 and the medical school three years later. Samuel

---

*Shortened version of the lecture delivered to the MSSVD at the Wright-Fleming Institute, St Mary's Hospital, London, 26 June 1981

Address for reprints: Dr R R Willcox, Tideway, Lonsdale Road, London SW13

Accepted for publication 8 September 1981



LOCK HOSPITAL SUPPORTED
THE LOCK HOSPITAL AT HYDE PARK CORNER IN THE TIME OF WATERLOO, 1815
TOTAL NUMBER OF BEDS IN 1815, 78

FIG 2 *Original Lock Hospital near Hyde Park Corner, London*

Armstrong Lane (1802-92) (fig 3), having been unable to get on the staff of St George's Hospital, was undoubtedly the chief person concerned in the founding of the medical school.[7]

The ties between St Mary's and the Lock hospitals were close (table I). Besides Samuel Lane, three others on the original staff of St Mary's were also on the staff of the Lock, including William Coulson, who founded the Hospital for Stone (St Peter's), which provided an early link between venereology and urology, and Samuel Lane's nephew, James Robert Lane.

SYPHILISATION

A striking example of international co-operation at the time took place at the Lock Hospital in 1865. A peculiar method of treatment, syphilisation, involved repeated inoculation of so-called syphilitic matter from other patients into those already having syphilis. It was originated by Auzias Turenne in Paris in about 1845 and resulted in much opposition and ridicule. It was, however, adopted for a time by Sperino of Turin with varied success and then on a large scale by Professor Carl Wilhelm Boeck (of Oslo), uncle of Professor Caesar Boeck of sarcoid and Boeck-Brusgaard material fame, who succeeded him. While on a professional visit to this country

FIG 3 *Samuel Lane*

TABLE I *Staff of St Mary's Hospital, who also served at the Lock Hospital*

Name	*Born*	*Died*	*Specialty*	*Appointed*
S A Lane	1802	1877	Surgeon	1854
W Coulson	1802	1887	Surgeon	1854
E Sieveking	1816	1904	Physician	1854
J R Lane	1825	1891	Surgeon	1854
G G Gascoyen	1830	1876	Surgeon	1864
W J Coulson	1834	1899	Surgeon	1865
H E Juler	1842	1921	Eye surgeon	1884
S Phillips	1851	1951	Physician	1884
J E Lane	1857	1926	Surgeon	1891

Boeck was invited by the Admiralty to state his views to a committee inquiring into the treatment and prevention of venereal diseases in the Army and Navy. He offered to initiate treatment by syphilisation if sufficient hospital accommodation could be made available. Beds were provided at the Lock Hospital and he came in August 1865 and stayed until the end of the year. Treatment was then continued by J R Lane and C G Gascoyen, who later published the results.[8]

The total number of inoculations made in 27 patients was 6993 and the mean number of those successful was 145 (range 66-296). A decade later in 1876—the year Harrison was born—Lane wrote in his Harveian *Lectures on Syphilis*[9] that if the treatment did all that was claimed it was worse than the disease. It entailed lifelong marking with cicatrices in return for doubtful advantages over other methods. It had been tried and found wanting; not surprisingly, as it is now obvious that it was chancroid which was being inoculated.

PROSTITUTION

The latter half of the century was undoubtedly the most active ever for the Lock Hospital; it was filled with female prostitutes from garrison towns through the operation of the Contagious Diseases Prevention Acts of 1864-9.[10] These gave legal approval to Britain's only serious attempt to regulate prostitution. Those found to be 'common prostitutes' in ultimately some 19 garrison towns were compelled to be medically examined; those infected were detained in a Lock hospital for up to three months. A special force of plain clothes officers was established to administer the Acts.[10]

Hardly were these laws established before the campaign against them grew (associated with the name of Josephine Butler); this had a marked influence on public opinion abroad. In 1875 an international federation was formed in Liverpool with the clumsy title 'The British, Continental, and General Federation for the Abolition of Government Regulation of Prostitution' (later changed to the International Abolitionist Federation). Its first international congress two years later was widely attended. The Acts were condemned by Parliament in 1883 and formally repealed in 1886.

Meanwhile, a movement for the suppression of the so-called white slave traffic grew in strength. Belgium had become the centre of the European market. Through the efforts of Mrs Butler, General Booth of the Salvation Army, the journalist W T Stead, and others this was exposed and international feeling aroused.[11] After a conference in Brussels in 1899 convened by the International Vigilance Association the French Government took the initiative in 1902 in calling an official meeting, which resulted in 1904 in the signing of an international agreement by delegates of 12 nations to monitor the situation. This led to the international convention which made the procuration of women and girls punishable by law.[12]

1900 – First World War

The first decade of the new century was marked by a cascade of new discoveries of the cause, diagnosis, and treatment of syphilis, which also brought that pioneer of immunology, Sir Almroth Wright (1861-1947), to St Mary's in 1902. Wright was a friend of Metchnikoff and particularly of Ehrlich.[13]

Harrison was taught by Wright, who had earlier held the chair of pathology in the Army Medical School, then at Netley, and regarded him as a great teacher.[14] Years later, during his first presidential address to the MSSVD he stated, 'I feel keenly the debt which I owe to the training in laboratory methods of attacking problems which I received for only too short a time from Sir Almroth Wright and later from my brother.'[15]

The new discoveries were quickly taken up by the inoculation department at St Mary's. Alexander Fleming (1881-1955), who had joined Wright's laboratory in 1906, was considerably involved. In 1909 he described the use of Hecht's modification of the Wassermann test, in which the serum to be tested furnished both complement and amboceptor but, with Scottish frugality, he modified it still further so that only a small quantity of serum was required.[16] In 1910, with Colebrook, he began to use salvarsan and in the following year they jointly described 46 cases in which this new drug—obtained from Ehrlich himself while on one of his visits to Wright's laboratory—had been injected in the wards of St Mary's.[17] They also used the drug in private practice and this contributed to the easing of Fleming's finances.[18 19]

L'OFFICE INTERNATIONALE D'HYGIÈNE PUBLIQUE

With the increasing number of scientific congresses which had followed in the wake of the steamship and train the need was gradually felt for more permanent bureaux to record and implement decisions. By 1912 there were 510 such bureaux covering trade, agriculture, postal services, and health.[20] The bureau for communicable diseases, l'Office Internationale d'Hygiène Publique, was established by international arrangement in Paris in 1907. Its bulletin was published monthly in French and consisted of reports concerning health legislation, some original articles, but mainly abstracts of papers relating to disease prevention—in many ways similar to the *Bulletin of* (now *Abstracts on*) *Hygiene* published by the Bureau of Hygiene and Tropical Diseases attached to the London School of Tropical Medicine.

ROYAL COMMISSION ON VENEREAL DISEASE

In 1913 the Royal Commission was set up to inquire into the true prevalence of venereal diseases in Britain, their effects on the health of the community, and how these could be alleviated or prevented.[21]

Two of St Mary's staff, the surgeon J E Lane (the son of James Robert Lane and also at the Lock Hospital) and Sir Malcolm Morris, the hospital's first dermatologist and an internationally renowned figure (whose interest in syphilis had resulted in a paper on salvarsan in the treatment of syphilitic glossitis in 1912 and who in the same year outlined in the *Lancet* a plea for the establishment of such a commission) served as members. James Pringle, dermatologist at the Middlesex Hospital, gave evidence among a number of prominent physicians which included Sir William Osler, and written statements were supplied by the recently promoted Colonel Harrison. Two international experts, Dr Svend Lomholt of Denmark and Dr Santoliquido of Italy both testified that if facilities were freely available there was no problem with 'quacks'.

The Commission reported in 1916 and its main recommendations were embodied in the VD Regulations of the following year. Harrison, starting at St Thomas's Hospital in 1919 and now technical adviser to the Ministry of Health, was naturally much involved in their implementation.[22]

SITUATION AT ST MARY'S HOSPITAL

Venereal diseases had previously been treated in the general surgical outpatients, but a separate clinic was established in 1916. Statistics were reported from 1917 onwards, and the returns of cases for 1919 and 1920 were signed by J E Lane. Although the pathology reports usually bore the signature of Captain A F Hayden, who served the clinic for many years, the half-yearly pathology return for 1919 was nevertheless signed by Fleming, who was obviously still concerned in this field.

When the Wassermann reaction became essential, Lane (by now senior surgeon at the Lock) had had Fleming appointed there to do the blood tests.[4] Although he first used his own modification of the test (Fleming's reaction) he later discarded this on finding it less reliable. He resigned from the Lock in 1919.

J E Lane also continued in charge at St Mary's until 1923 when he sustained a fractured femur when knocked down by a bus in Regent Street. The fracture did not heal and he died after three years in the Star and Garter Home at Richmond. A picture survives of him during the First World War, when for a time he was in charge of the American Military Hospital at Paignton in Devon, his most distinguished international role (fig 4).

FIG 4 *J E Lane*

Between the World Wars

FORMATION OF THE LEAGUE OF NATIONS

As envisaged by the Treaty of Versailles the League of Nations was formed in 1919 with financial, economic, and transit committees and a technical health committee. The last was concerned with the standardisation of drugs and sera. It was also responsible for the exchange of knowledge on communicable diseases and arranged missions to the Middle and Far East, Russia, and South-east Europe. In addition, by the generosity of the Rockefeller Foundation, it was made possible for health administrations to send officers to study methods in other countries, participating in practical work and meeting afterwards to discuss their findings in Geneva.

The Health Organisation also maintained an epidemiological intelligence service publishing worldwide data on epidemic diseases. It was noted, however, that the pre-existing bureau showed no eagerness to surrender its autonomy when maintaining contact with the League of Nations office.[23]

STANDARDISATION OF LABORATORY TESTS

In the period after the First World War, after many previous attempts, effective flocculation tests for syphilis were being introduced which were much simpler than complement-fixation procedures. Each test had its own supporters and it was difficult to know which was best.

At the North European Red Cross Congress in Copenhagen in May 1921 Harrison put forward the suggestion of international proficiency testing, which was taken up keenly by Dr Th Madsen, director of the State Serotherapeutic Institute in Copenhagen. He was president of the health section of the League of Nations and may have been thinking on similar lines.[24] In November of that year at an international conference under the auspices of the Standardisation Committee of the League of Nations it was arranged that the same sera should be tested in a number of European laboratories by three flocculation procedures following the precise techniques of their authors and by the local modification of the Wassermann reaction.

Harrison, now at the Ministry of Health, arranged for Dr E J Wyler (who worked at the Ministry's Public Health Laboratory, the forerunner of what is now the Reference Laboratory, and who would have used the Harrison-Wyler modification of the Wassermann) to go to Heidelberg, Oxford, and The Hague to master each author's requirements.

The results were presented in Paris in 1922 and a further conference was convened in Copenhagen the following year to arrange for the testing of the same approximately 530 sera by other European laboratories. At a later conference, held in Montevideo in 1928 and attended by both Wyler and Reuben Kahn, some 996 sera and 200 samples of cerebrospinal fluid were tested by South American workers.[25] The newer Kahn test emerged the equal of the Wassermann.

These conferences were considered by Harrison to be 'great disillusioners'. They clearly showed that not only did workers not obey agreed protocol but meaningful comparisons of results between laboratories were not yet possible and that some of the tests other than the Kahn were 'sadly wanting in reliability'.[24]

FORMATION OF THE INTERNATIONAL UNION

The Union Internationale contre le Péril Vénérien (IUPV), as it was then called, was formed in Paris in 1923; our own national society, the MSSVD, was established in London during the previous year. The *British Journal of Venereal Diseases* made its first appearance in 1925.[26]

BRUSSELS AGREEMENT

Apart from prostitutes the largest high-risk group in those days was merchant seamen. The need for making free treatment facilities available to seafarers regardless of nationality, and of standardising control methods in ports, had been appreciated for many years. Indeed Denmark had been the first country to move in this direction as far back as 1802. The newly established (1919) League of Red Cross Societies discussed the problem in Geneva, Copenhagen, Prague, and Paris in 1920-1 by which time Britain was already providing free treatment to sailors regardless of nationality.

When the Brussels agreement finally emerged in 1924, to be administered by l'Office Internationale d'Hygiène Publique in Paris, it was the culmination of the post-war activities of a number of international organisations, including the International Labour Office (ILO) (also established in 1919), the Maritime Commission, the Health Organisation of the League of Nations, and the Union Internationale contre le Péril Vénérien. It was signed by 14 countries, ratified by 11, and the number adhering to it later increased to 56.

LEAGUE OF NATIONS COMMITTEE OF EXPERTS

Reponding to the need for authoritative recommendations for the treatment of syphilis the Health Organisation of the League of Nations convened a Committee of Experts on Syphilis and Cognate Subjects in 1928 under the chairmanship of Professor J Jadassohn, then director of the Dermatology Clinic at Breslau, and a member of the League's Health Committee, as president. Other members included Dr John Stokes of Philadelphia, Professor Rasch of Copenhagen, Professor Louis Quéyrat (of erythroplasia fame, then president of the Ligue Nationale Française contre le Péril Vénérien), Dr Th Madsen, and Colonel Harrison. Professor H Gougerot of Paris and Dr Svend Lomholt of Copenhagen joined later.

The experts recommended that a retrospective cooperative study should be carried out of the cases of syphilis treated in different countries using cards designed for the purpose to provide a basis for the recommendations. Some 25 200 cards were ultimately obtained from Denmark, France, Germany, Britain, and the USA, in which countries the study was concentrated. Britain contributed just over 3500 with a very wide distribution (table II). St Mary's and the Lock were regrettable absentees. The analysis was entrusted to Professor Hans Martenstein of Dresden who concentrated on the 13 200 cards relating to secondary syphilis, of which nearly 2000 had to be discarded, as insufficient laboratory tests had been used. His findings were not forthcoming until November 1934 and the full report of the Committee of Experts was published in the *Quarterly Bulletin of the Health Organization* in March 1935 and was reviewed extensively by Harrison.[27]

TABLE II *League of Nations syphilis inquiry: "top ten" of 28 British participating clinics (3571 cards)*

St Thomas's, London	955	Wolverhampton	195
Guy's, London	300	British Army	186
Newcastle	247	Brighton	136
Dundee	243	Portsmouth	134
Bradford	230	Edinburgh	111

The Committee laid down the principles to be followed in arsenic and bismuth therapy, most of which have considerable relevance today. The data, however, did not allow a clear decision on the relative merits of the methods used.

Meanwhile, some 50 yards from here, in the year the Committee first sat, an event occurred which was of universal significance and which was to render its deliberations largely redundant. While Captain Hayden still performed the blood tests for the clinic, Fleming had discovered penicillin.[28]

POST-WAR YEARS AT ST MARY'S

After the death of J E Lane the long association between the staff of St Mary's and the Lock ended. The Lock staff were now mainly surgeons with a urological background and included the redoubtable J Johnston Abraham (1876-1963), an almost exact contemporary of Harrison, with whom the Lock came to be so closely identified. St Mary's students went to the Lock, however, for teaching, as later did I. At St Mary's, the venereal disease clinic continued to be run by surgeons, namely C W Gordon Bryan and R M Handfield Jones, acting on Bryan's behalf.

A crisis came in 1932, the year after I came as a student, when the London County Council indicated that they might not continue their financial support unless the clinic remained open all day with a medical officer in attendance, that it was under the control of one person who should have charge of both syphilis and gonorrhoea, and that it was conducted in suitable premises especially designed for the purpose.

The LCC's conditions were met and on 1 June 1933 Dr G L M McElligott (1897-1972) (fig 5) who had been clinical assistant with Colonel Harrison at St Thomas's Hospital, and more recently in charge of the clinic at Stoke on Trent, was appointed director on a salary of £400 a year. Arthur Porritt, who later as Lord Porritt served as Governor-General of New Zealand, was made assistant director. In 1936 he was succeeded by another surgeon, Mr A J Cokkinis, who had earlier been a surgical registrar at the Lock.

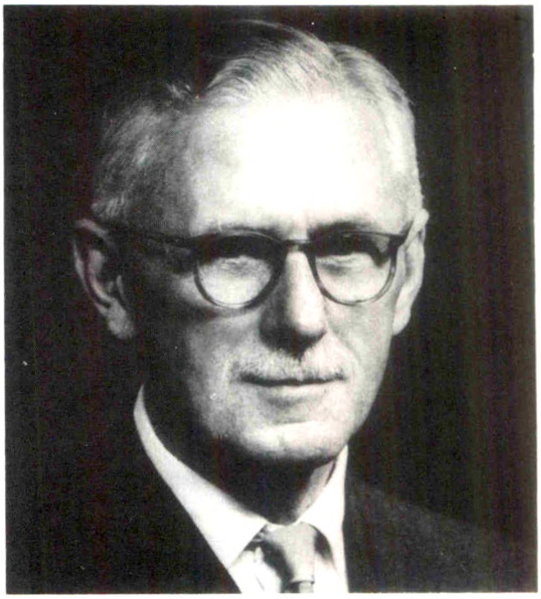


FIG 5 *G L M McElligott*

Accommodation was found in the basement of the old building and was enlarged by extending the premises into the yard behind; these were to serve as a VD clinic for over 40 years until, with Dr Jefferiss and myself, it was moved into its present purpose-built premises some nine years ago.[29]

Dr McElligott established St Mary's as the first British full-time venereal disease clinic, although it was not until after the war that this became a reality.

INTERNATIONAL VD STUDY GROUPS

Another international pattern was set in 1935 when an investigatory group from New York visited Denmark, Sweden, and Britain to study antivenereal disease measures in these countries. In their report,[30] which recognised the value of free venereal disease services in Europe, Britain was compared somewhat unfavourably with Sweden and Denmark where laws on compulsory treatment, punishment for transmission of infection, and prevention of marriage of infected persons were in force.

Harrison was not unnaturally critical of this report, as like was not compared with like in the quoted statistics, and arranged an official mission of his own in 1937 to visit Denmark, Norway, Sweden, and Holland. One of its four members was Dr Margaret Rorke, consultant venereologist at the Royal Free Hospital. The official report, a model of its kind, had Harrison as its first author.[31] His own account of this mission, given to the MSSVD to inaugurate his unique second term as president,[32] concluded that the Scandinavian antivenereal laws worked smoothly, did not lead to concealment of disease, and had undoubtedly led to a reduction in the incidence of syphilis, although despite them, that of gonorrhoea was still very high. He calculated too that the incidence of new cases of syphilis in England and Wales was "very little if any" greater than in Denmark.

RHINE BOATMEN

The need for co-operation between the countries bordering on the Rhine waterway and the establishment of a co-ordinated system for the treatment of venereal diseases for the Rhine boatmen was discussed by the Union Internationale contre le Péril Vénérien at their General Assembly in Amsterdam in 1936. An international sub-committee to co-ordinate such efforts was formed.[33]

TRAFFIC IN WOMEN AND CHILDREN

The international convention for the suppression of the white slave traffic of 1910 had been concluded in 1921 under the auspices of the League of Nations with its new title 'Traffic in women and children' and a special body of experts was appointed to monitor the subject.[12] A further convention in 1933 aimed at the suppression of such traffic in women of full age. By 1937 a draft convention was prepared by the League with the object of securing concerted international action for the abolition of licensed houses and for the prosecution of those managing a brothel or exploiting the prostitution of others. It was planned that this should be concluded at an international convention in 1940 but the Second World War prevented this. The matter was taken up again by the Economic and Social Council of the United Nations (UNESCO) after the war and finally led to the adoption of the Convention for the Suppression of the Traffic in Persons and of the Exploitation of Prostitution of Others.[34] The League's active interest in prostitution nevertheless continued. In the middle of the Second World War its advisory committee on social questions published a report on the prevention of prostitution particularly with regard to minors.[35]

Second World War

WAR TIME AT THE LOCK

The outbreak of war, however, brought the Lock Hospital a flourishing new but terminal role as the Military Isolation Hospital, Harrow Road, for the treatment of both skin (there was much scabies) and venereal diseases. The VD specialist, who later became a dermatologist, was Major James Marshall, formerly pre-war registrar at the same hospital, while at the War Office Harrison's St Thomas's pathologist, Brigadier T E Osmond, was adviser.

The chapel was deconsecrated and on Sundays its gallery no longer housed the uniformed female patients deliberately obscured from public view, being used only for an occasional ENSA party, members of which would look quizzically when they learnt that the boys in blue they were going to entertain had been wounded in an unusual place.

The wards were filled with patients with epididymitis and arthritis, but above all with hepatitis which affected 50% of those treated for syphilis before it was established it was transmitted by syringes, and sterilisation by heat was imposed.[36]

After the war, the Lock never survived as a hospital; it was used for some years for administrative offices in the new National Health Service but is now boarded up and partially derelict.

DEVELOPMENT OF PENICILLIN

Meanwhile in the USA, thanks to Florey, penicillin was now in production.[16 17] Mahoney and his colleagues had treated four seamen with syphilis at Staten Island.[37] Back at St Mary's Jack Suchet was tentatively treating a variety of cases of venereal disease with Fleming's penicillin; they both addressed the MSSVD on the subject in 1944.[38 39]

As the war ended Harrison, now nearly 70 years old but not yet retired from the Ministry—and who had earlier endeavoured to organise consistent arrangements for the treatment of venereal diseases among the merchant seamen of the various allies,[14] was visiting the Lock Hospital to inquire into the results of a trial he had organized of a British formulation of penicillin in oil-beeswax in the treatment of gonorrhoea.[40]

Early in 1946 a large conference of research workers was called in Washington to present and discuss the already considerable accumulated experience of penicillin therapy of syphilis in the USA. I attended this meeting and its committees as the British Army observer and was able to present the findings in some detail to the MSSVD very shortly after my return.[41]

CREATION OF THE WORLD HEALTH ORGANISATION

The United Nations replaced the League of Nations in 1945. The constitution of the new World Health Organisation was signed by the representatives of 61 countries at an International Health Conference held in New York in 1946. Pending ratification by member states an interim commission was formed until the organisation was fully established in 1948.

Dr Thorstein Guthe, an Olympic oarsman and Norwegian naval physician (fig 6) was on the secretariat of the commission and responsible for venereal infections. He had observed Mahoney's original four patients with syphilis who were treated with penicillin and his very considerable energy, astuteness, and organising capacity were fired with an obsessional enthusiasm for venereology which was highly contagious to all exposed to it.

Meanwhile the Union Internationale contre le Péril Vénérien convened an executive committee meeting in Paris in 1946 and the first post-war general assembly in the same city the following year. Both Colonel Harrison (a technical counsellor) and Dr Guthe attended this meeting (fig 7), at which penicillin therapy was discussed and Guthe outlined the future plans of the WHO.[42]

ACTIVITIES OF THE WHO

The new World Health Organisation was quickly established in the Palace of the former League of

FIG 6 *Thorstein Guthe*

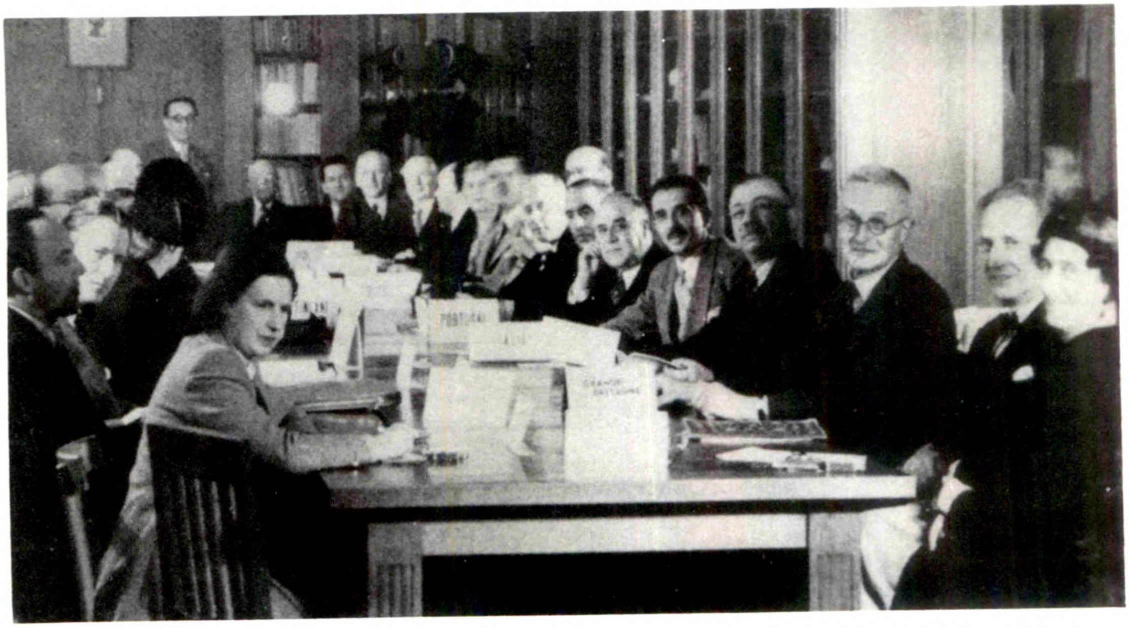


FIG 7 *UIPV executive committee meeting, Paris 1947; Thorstein Guthe (seated third on the left of the table) and Colonel Harrison (seated third on the right)*

Nations in Geneva and not surprisingly followed several familiar paths in its approaches to new problems. It absorbed a number of pre-existing organisations including l'Office Internationale d'Hygiène Publique and with it the administration of the Brussels Agreement.

WHO/VDT UNIT

At Geneva the small VDT Unit under Guthe, aided by one physician (at first by the late Dr Frank Reynolds of the USA followed by Dr C J Hackett of Britain), one personal non-medical assistant, and two secretaries, plus an occasional consultant (which happily included me) set about its self-appointed task of helping others to raise the standards and effectiveness of venereal disease control throughout the world. This was achieved by stimulating, facilitating, and co-ordinating national venereal disease programmes, exchanging up-to-date scientific information (including training), and fostering research into all aspects of our specialty. This particularly involved co-operation with other established international and non-governmental agencies as well as with other units within the Organisation itself. First priority in these activities was the venereal and the non-venereal treponematoses in economically underdeveloped areas of high prevalence.[43] An expert committee on venereal infections (treponematoses being soon added), with a sub-committee on the serological aspects, was then convened; an international serological laboratory was established at Copenhagen and a WHO international research laboratory at Baltimore. A more widely based WHO advisory panel of experts was gradually assembled.

EXPLOITATION OF THE POTENTIALITIES OF PENICILLIN

A first objective was to exploit to the full the potentialities of penicillin. One of the problems in these early days was to "sell" the new antibiotic treatment for syphilis to European dermatovenereologists entrenched and enriched by long-term treatments with arsenic and heavy metals. This resistance was very largely overcome by sending a WHO study commission to the USA[44] (on which Dr Sydney Laird served) and at two regional symposia in Helsinki and Paris in 1950.[45]

Workers in developing countries and areas of high prevalence of treponemal disease were more receptive and many WHO-assisted mass campaigns based on penicillin (fig 8) were organised, starting with venereal syphilis in Poland, endemic syphilis in Yugoslavia and Iraq, and yaws in Thailand, Haiti, the Philippines and Indonesia, in which millions of patients were examined and treated (fig 9).[46] Sufficient experience had accumulated from the yaws

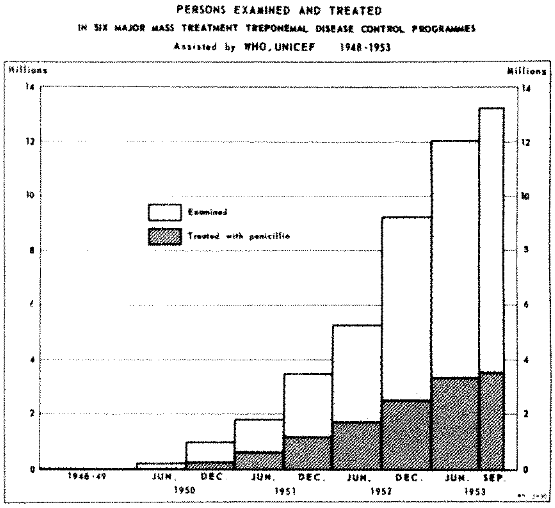


FIG 9 *Increasing numbers treated in WHO campaigns*

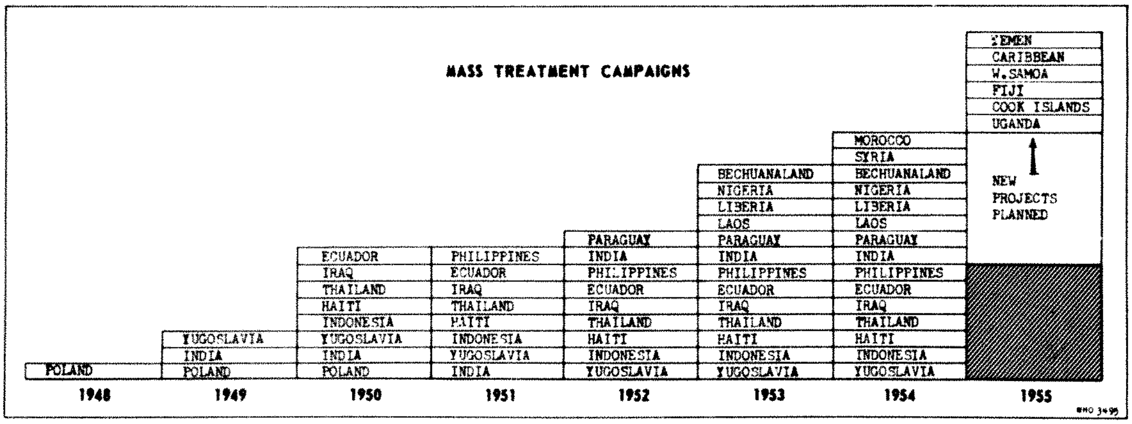


FIG 8 *WHO mass treatment campaigns*

campaigns for international symposia to be held in Bangkok in 1952[47] and in Ibadan in 1955,[48] both of which I had the privilege of attending.

EXPERT COMMITTEES

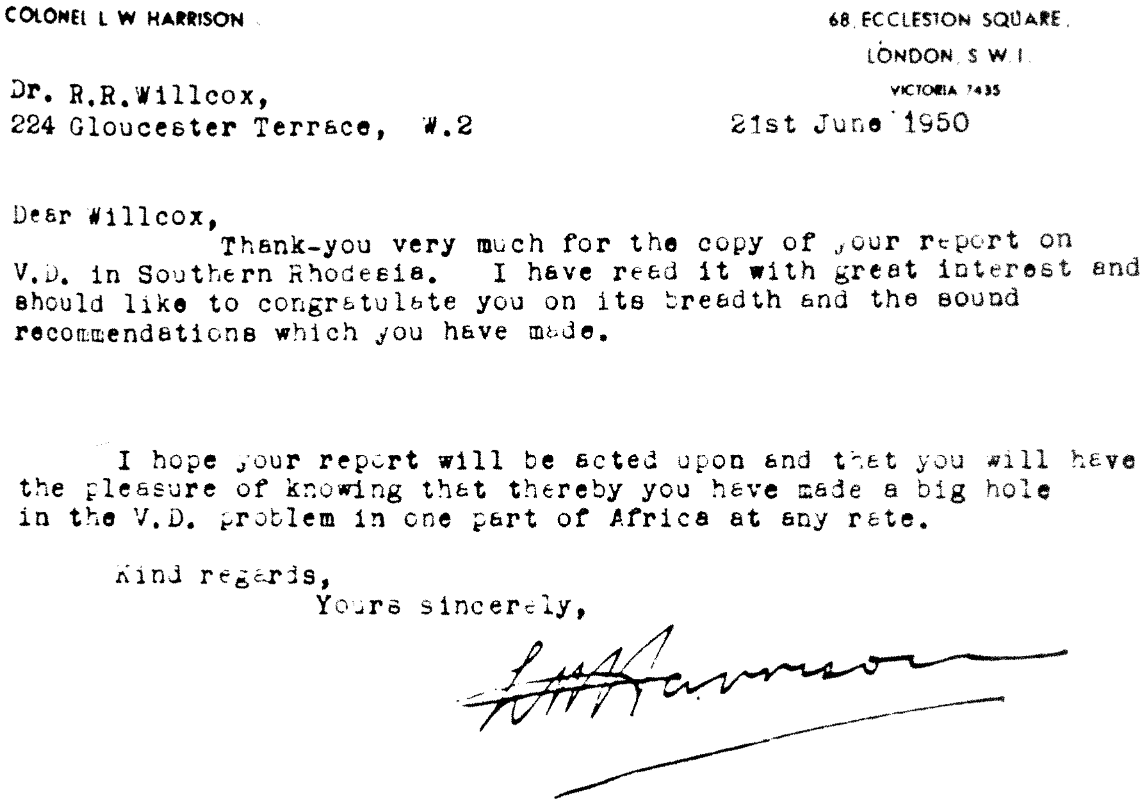
The momentum was maintained by repeated meetings of the expert committee in 1948 in Geneva and again in Paris; in 1949 in Washington[43] (Dr McElligott, who had succeeded Colonel Harrison at the Ministry of Health in London, was then a member), in 1952 in London,[45] and again in 1959[49] in Geneva. A sub-committee on serological aspects met in Washington,[50] Paris,[51] and Copenhagen[52] over this period. (The late Dr I N Orpwood Price, director of the Venereal Disease Reference Laboratory attended these).

Colonel Harrison, by now recently retired, was a member of the WHO expert advisory panel on serology and laboratory aspects and maintained a continuing interest in the international scene: Guthe visited him when in London. In 1950, in response to receiving the report of a venereal disease survey, undertaken by me for the then Government of Southern Rhodesia[53] during six months leave in what is now Zimbabwe, Harrison replied in a closely typed two-page letter from which, not just for reasons of vanity, the first and last paragraphs are reproduced (fig 10). The hopes expressed in the closing lines were not fulfilled, however, as it appears that in many respects the overall situation is in fact little changed today.[54 55]

ACHIEVEMENTS OF THE WHO

Broad measures

The achievements of the WHO in the first 10-15 years were considerable. Standard specifications of procaine penicillin in aluminium monostearate (PAM) were defined, and minimum treatment schedules for the treatment of syphilis and the non-venereal treponematoses based on this preparation were formulated.[45] At Copenhagen reference preparations of cardiolipin and lecithin were made available, WHO-directed interlaboratory testing of freeze-dried sera organised (with reference centres in Denmark and the USA collaborating), and standard reference sera provided on demand. These activities replaced the



COLONEL L W HARRISON

68, ECCLESTON SQUARE,
LONDON, S.W.1.
VICTORIA 7435

Dr. R.R.Willcox,
224 Gloucester Terrace, W.2

21st June 1950

Dear Willcox,

Thank-you very much for the copy of your report on V.D. in Southern Rhodesia. I have read it with great interest and should like to congratulate you on its breadth and the sound recommendations which you have made.

I hope your report will be acted upon and that you will have the pleasure of knowing that thereby you have made a big hole in the V.D. problem in one part of Africa at any rate.

Kind regards,
Yours sincerely,

L W Harrison

FIG 10 *Colonel Harrison's letter to Dr R R Willcox*

pre-war serological conferences of the League of Nations. At Baltimore basic research into the biology of treponematoses was undertaken, while in Guatemala a serological laboratory training centre was concerned in training and the standardisation of techniques. Also through the WHO the introduction of the *Treponema pallidum* immobilisation (TPI) test was guided in some 41 laboratories, mostly in Europe and the USA.[56]

Maritime aspects

The WHO formed a study group to clarify the Brussels Agreement (to which 67 authorities had now acceded or ratified while 30 others notified the WHO that free treatment was available in certain ports),[57] introduced a new personal carnet, and published directories of VD treatment centres in ports.[58] A Port Demonstration Centre was established in Rotterdam and used for the training of physicians.

An international antivenereal disease commission of the Rhine was established in 1949 to cater for the needs of the boatmen on this river,[33] [58] but the need for such a specialised organisation soon diminished and it was disbanded in 1953.

Consultants and fellowships

A number of other demonstration and training projects were instituted (fig 11) and other consultants were sent to project areas. Drs K R Hill, S M Laird, F R Curtis, G W Csonka, and the late 'Gobi' Jones (then working in the St Mary's clinic) were among those from this country.

Numerous fellowships (fig 12) were awarded for the training of physicians or key laboratory personnel. One brought the late C W Chacko (who was later killed in an aircrash while on a WHO assignment) to St Mary's to establish the TPI test before taking it back to Bombay and later to Madras.

Lecture courses were arranged through the regional offices, such as those in Beirut and Rotterdam (the WHO regional office for Europe in those days was also in the Palais des Nations, Geneva), and a WHO travelling VD seminar spent some weeks studying methods in the USSR.

Publications

Monographs or similar volumes were published on cardiolipin antigens,[59] yaws[60] and yaws control,[47] endemic syphilis,[61] donovanosis,[62] venereal disease legislation,[63] treponematoses as a world problem,[46] and their biology and research aspects,[64] yaws nomenclature,[65] and, in 1960, its differential diagnosis.[66] In addition special numbers of the WHO *Bulletin* were organised and studies were made of penicillin reactions.[67] Throughout, a long line of cyclostyled VDT/documents, which included much not yet published research, flowed from Geneva to panel members and others interested on a worldwide basis.

BRITISH CO-OPERATIVE CLINICAL GROUP

Guthe stimulated me in 1952 to participate in forming the British Co-operative Clinical Group to undertake a study of the results of the treatment of syphilis with penicillin plus metallotherapy, which

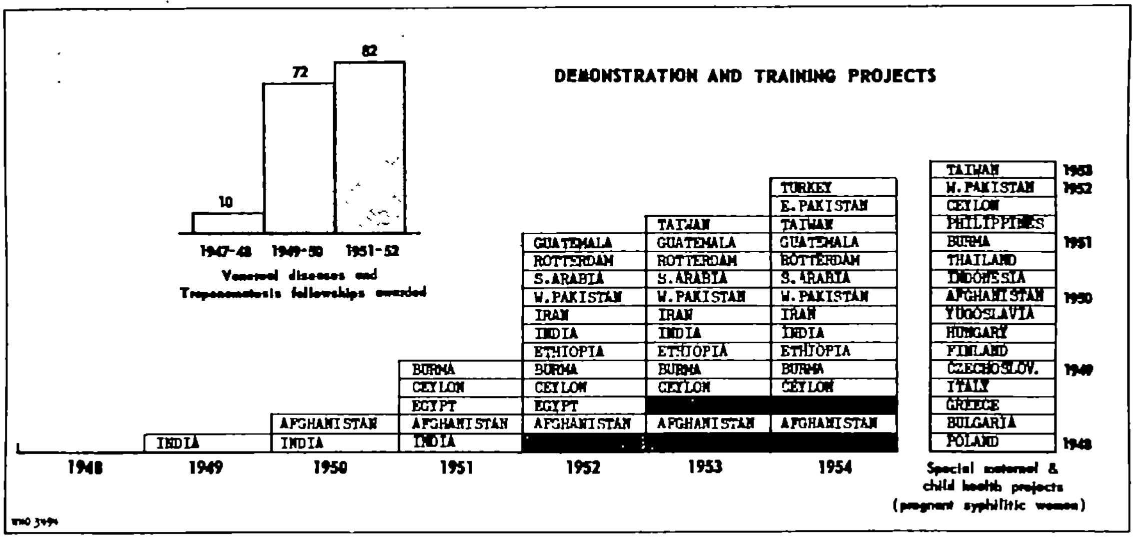


FIG 11 *WHO demonstration and training projects*

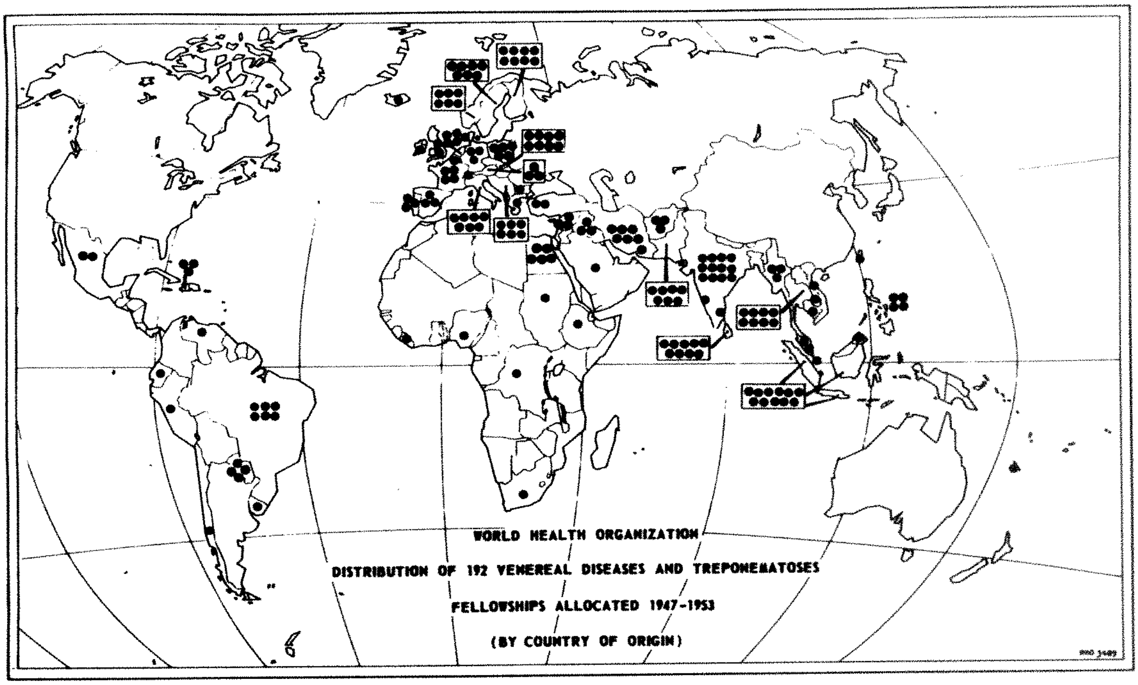

WORLD HEALTH ORGANIZATION
DISTRIBUTION OF 192 VENEREAL DISEASES AND TREPONEMATOSES
FELLOWSHIPS ALLOCATED 1947-1953
(BY COUNTRY OF ORIGIN)

FIG 12 *WHO fellowships allocated, 1947-53*

had previously been used in Britain. Some 104 clinics participated in this study, and 9600 cards relating to cases of syphilis treated in 1946 were sent to the WHO. But like the League of Nations study 25 years before, it foundered in the hands of the statisticians and no firm conclusions could be drawn. The group also co-operated with the WHO in studies of penicillin blood concentrations and in the use of the newly arrived benzathine penicillin.

OTHER INTERNATIONAL ACTIVITIES

As syphilis became less prevalent, and the success of the mass campaigns was being evaluated, the problem of gonorrhoea and of non-specific urethritis was increasing.

The International Union (IUVDT), which had soon followed the WHO by adding 'and Treponematoses' to its title (and dropping Péril), held the first symposium on non-gonococcal urethritis in Monaco in 1954. This was attended by Dr A Cavaillon (1887-1967), with whom the Union had so long been associated, and by Dr A H Harkness with whose name the role of chlamydia in non-gonococcal urethritis will always be remembered.[68]

The WHO organised broader based meetings in Tokyo in 1958 and with the IUVDT and United States Public Health Service in Washington in 1952 and again in 1962. Because of the alarm expressed by the expert committee in 1959 at the evident failure to control gonorrhoea,[50] the WHO established an International Gonococcus Reference Laboratory in Copenhagen, convened a separate expert committee on gonococcal infections in Geneva in 1962,[69] and planned for a meeting on *Neisseria* research to be held two years later.[70]

Our own MSSVD took wings with the first of its many overseas meetings in Paris in 1961 and its previous perhaps somewhat insular attitude was further broadened by the election of Mr Ambrose King as president of the IUVDT in 1962.

Conclusions

All the events which have been described occurred before the death of Harrison in 1964. Not to be forgotten in this story are the contributions of numerous other bodies international and national, governmental and non-governmental, including the Armed Forces, British Council, the Rockefeller Foundation, medical societies of other disciplines (dermatology, microbiology, epidemiology, behavioural sciences), and many individuals such as the late Dr J E Earle Moore of Baltimore, who have materially aided the international exchange of scientific information and views.

Recommendations are one thing but their implementation is another, even if they are backed by legislation. Decrees, laws, regulations, or conventions, whether local, national, or international, are as nought unless heavily backed by the voluntary co-operation of those individuals expected to enforce them. Today, with the current multiplicity of international meetings involving a relatively small group of venereal disease specialists the opportunities for seeking such co-operation on a world-wide scale have, in theory, never been better. Whatever the problems, however, while the pace has undoubtedly quickened, it can safely be predicted that many international efforts made for their solution will strike familiar notes on the gongs of the past.

I am grateful to the librarians of the Royal College of Surgeons, the British Medical Association, the London School of Tropical Medicine and Hygiene, and St Mary's Hospital for their repeated assistance.

Figs 1, 2, and 4 are reproduced by kind permission of the Royal College of Physicians, fig 3 by kind permission of Heinemann Medical Books Ltd, and figs 8, 9, 11, and 12 by kind permission of the World Health Organisation. I am grateful to Dr P N Cardew of the Department of Audiovisual Communication, St Mary's Hospital, for providing fig 5.

References

1. Willcox RR. Venereal disease in the Bible. *Br J Vener Dis* 1949; **25**:28-33.
2. Schokking CPh. The Brussels Agreement in retrospect. In: *Maritime Venereal-Disease Control: Selected Lectures, Rotterdam 1953-1954*. Geneva: WHO Regional Office for Europe, 1956.
3. Abraham JJ. The early history of syphilis. *Br J Surg* 1944; **32**: 225-37.
4. Abraham JJ. *Surgeon's Journey*. London: Heinemann, 1957.
5. Farey W. The Lock Charity. *Westway* 1978; March: 11.
6. Rugg-Price A. Paddington Workhouse and Infirmary 1846-1900. *Westway* 1974; June: 10-11.
7. Cope Z. *The History of St. Mary's Medical School, London*. London: Heinemann, 1954.
8. Lane JR, Gascoyen GG. Record of cases treated in the Lock Hospital by syphilization. *Med Chir Trans* 1867; 281-328.
9. Lane JR. *Lectures on Syphilis:* delivered at the Harveian Society, December 1876. London: J & A Churchill, 1878.
10. Scott GR. *A History of Prostitution from Antiquity to the Present Day*. London; T Werner Laurie, 1936.
11. Bullough V, Bullough BL. *A History of Prostitution*. New York: University Books, 1964.
12. League of Nations. *Report of the Special Body of Experts on Traffic in Women and Children*. Geneva: 1927; 6-7.
13. Colebrook L. *Almroth Wright—Provocative Doctor and Thinker*. London: Heinemann Medical Books, 1954.
14. Harrison LW. Half a lifetime in the management of venereal diseases. From chaos to order. *Med Illustr* 1949; July: 318-24; Aug: 376-85.
15. Harrison LW. The value of a scientific outlook to the worker in venereal diseases. *Br J Vener Dis* 1925; **1**: 81-5.
16. Fleming A. Demonstration of a simple method of serum diagnosis of syphilis by the "complement deviation" method. *Proc R Soc Med,* 1909; **2**: 220-5.
17. Fleming A, Colebrook L. On the use of salvarsan in the treatment of syphilis. *Lancet* 1911; 1631-4.
18. Maurois A. *The Life of Sir Alexander Fleming. Discoverer of Penicillin.* London: Jonathan Cape, 1959.
19. Crowther JG. *Alexander Fleming.* Heron Books, 1971.
20. Anonymous. International Bureaux. *Encyclopedia Britannica,* 1946; **12**: 515.
21. Royal Commission on Venereal Diseases. *Final Report of the Commissioners.* London: HMSO, 1916.
22. Harrison LW. The public health services and venereal diseases. *Br J Vener Dis* 1925; **1**: 12-22.
23. Anonymous. League of Nations. *Encyclopedia Britannica* 1946; **13**: 829-44.
24. Harrison LW. Introductory remarks to demonstration of flocculation tests. *Br J Vener Dis* 1931; **7**: 89-97.
25. League of Nations Health Organization. *Report of the Laboratory Conference on the Serodiagnosis of Syphilis,* convened at Montevideo by the Institute for the Prevention of Syphilis of Uruguay, 15-26 September 1930. Geneva, 1931.
26. Harrison LW. History of the Medical Society for the Study of Venereal Diseases (with a history of the Scottish Branch by R Forgan), *Br J Vener Dis* 1961; **37**: 2-32.
27. Harrison LW. The treatment of early syphilis. The enquiry and recommendations of the League of Nations Health Organization. *Br J Vener Dis* 1935; **11**: 69-90.
28. Fleming A. On the antibacterial action of cultures of a penicillium with special reference to their use in the isolation of *B influenzae. Br J Exper Pathol* 1929; **10**: 226-36.
29. Jefferiss FJG, Willcox RR, James DT. *The Hospital and Health Services Review* 1974; **70**: 9-13.
30. Rice JL, Kalinski DJ, Farmer TP, Parran T. (report compiled by Clarke CW). Control of syphilis and gonorrhoea in Scandinavian countries and in Great Britain. *Amer J Syph* 1936; **20**: 1-63. (see abstract by Harrison LW. *Bull Hyg* 1936; **11**: 910).
31. Ministry of Health. *Report on Anti-venereal measures in certain Scandinavian Countries and Holland.* London: HMSO, 1938.
32. Harrison LW. Anti-venereal measures in Denmark and Sweden. *Br J Vener Dis* 1939; **15**: 1-17.
33. Hermans EH. The Rhine River Commission. In: *Maritime Venereal Disease Control,* Geneva: WHO Regional Office for Europe, 1956: 21-26.
34. Willcox RR. *Textbook of Venereal Infections and Treponematoses,* 2nd ed. London: Heinemann 1964: 424-5.
35. League of Nations Advisory Committee on Social Questions. *Prevention of Prostitution.* A study of measures adopted or under consideration particularly with regard to minors. Geneva; League of Nations, 1943.
36. Willcox RR. The effects of syringe-transmitted jaundice on the outcome of the treatment of early syphilis. *Br J Vener Dis* 1947; **23**: 121-3.
37. Mahoney JF, Arnold DC, Harris A. Penicillin treatment of early syphilis. A preliminary report. *Venereal Disease Information* 1943; **24**: 355-7.
38. Fleming A. Penicillin in venereal disease, part I. *Br J Vener Dis* 1944; **20**: 133-6.
39. Suchet J. Penicillin in venereal diseases, part II. *Br J Vener Dis* 1944; **20**: 136-8.
40. Ministry of Health. *On the State of the Public Health during Six Years of War.* London; HMSO 1946; 66-73.
41. Willcox RR. Current trends in the treatment of venereal disease in the United States of America. *Br J Vener Dis* 1946; **22**: 102-11.
42. Union Internationale contre le Péril Vénérien. Assemblée Générale, Paris 20-25 October 1947. *Texte de Rapports et Compte Rendu des Débats,* Paris, 102-105.
43. World Health Organization. Expert Committee on Venereal Infections: Report of the Third Session. *Tech Rep Ser* No 13, Geneva; WHO, 1950.
44. World Health Organization. Venereal Disease Control in the USA: report of the WHO Study Commission. *Tech Rep Ser* No 15, Geneva; WHO, 1950.
45. World Health Organization. Expert Committee on Venereal Infections and Treponematoses. Fourth report. *Tech Rep Ser* No 63, Geneva; WHO, 1953.
46. Guthe T, Willcox RR. Treponematoses a World Problem. *Chronicle of the WHO* (special issue) 1954; **8**: 37-114.
47. World Health Organization. First International Symposium on Yaws Control 1953. *Monograph Series* No 15, Geneva; WHO, 1953.

48. Report of International Conference on Control of Yaws, Nigeria 1955. *J Trop Med Hyg* 1957;60:1-24.
49. World Health Organization. Expert Committee on Venereal Infections and Treponematoses: fifth report. *Tech Rep Ser* No 190, Geneva; WHO, 1960.
50. World Health Organization. Expert Committee on Venereal Infections: report on the First Session of the Sub-committee on Serology and Laboratory Aspects. *Tech Rep Ser* No 14, Geneva; WHO, 1950.
51. World Health Organization. Expert Committee on Venereal Infections and Treponematoses: report on the Second Session of the Sub-committee on Serology and Laboratory Aspects. *Tech Rep Ser* No 33, Geneva; WHO, 1951.
52. World Health Organization. Expert Committee on Venereal Infections and Treponematoses. Sub-committee on Serology and Laboratory Aspects: Third Report. *Tech Rep Ser* No 79, Geneva; WHO, 1954.
53. Willcox RR. *Report of a Venereal Disease Survey of the African in Southern Rhodesia.* Salisbury: Southern Rhodesian Government; 1949.
54. Latif AS. Sexually transmitted disease in clinic patients in Salisbury, Zimbabwe, *Br J Vener Dis* 1981;57:181-3.
55. Bingham JS. Zimbabwe: land of hope and . . . *Br J Vener Dis* 1981;57:184-6.
56. World Health Organization. *The Medical Research Programme of the World Health Organization 1958-1963:* report by the Director General, Geneva; WHO, 1964;77-91.
57. World Health Organization. The Agreement of Brussels 1924 respecting facilities to be given to merchant seamen for the treatment of venereal diseases. Report of a Study Group. *Tech Rep Ser* No 50, Geneva; WHO, 1958.
58. World Health Organization. *World Directory of Venereal Disease Treatment Centres at Ports,* 2nd ed, Geneva; WHO, 1961.
59. Pangborn MC, Almeida JO, Maltaner F, Silverstein AM, Thompson WR. Cardiolipin antigens. *WHO Monogr Ser* No 6, Geneva; WHO, 1951.
60. Hill KR, Kodijat R, Sardadi M. Atlas of framboesia. *WHO Monogr Ser* No 5, Geneva; WHO, 1951.
61. Grin EI. Epidemiology and control of endemic syphilis. *WHO Monogr Ser* No 17, Geneva; WHO, 1953.
62. Rajam RV, Rangiah PN. Donovanosis (granuloma inguinale, granuloma venereum). *WHO Monogr Ser* No 24. Geneva; WHO 1954.
63. World Health Organization. *Venereal Diseases. A Survey of Existing Legislation.* Geneva; WHO, 1956.
64. Turner TB, Hollander DH. Biology of the treponematoses. *WHO Monogr Ser* No 35, Geneva; WHO, 1957.
65. Hackett CJ. An international nomenclature of yaws lesions. *WHO Monogr Ser* No 36, Geneva; WHO, 1957.
66. Hackett CJ, Lowenthal LJA. Differential Diagnosis of Yaws. *WHO Monogr Ser* No 45, Geneva; WHO, 1960.
67. Guthe T, Idsøe O, Willcox RR. Untoward penicillin reactions. *Bull WHO,* 1958;19:427-501.
68. Harkness AH. *Non-gonococcal Urethritis.* Edinburgh; Livingstone, 1950.
69. World Health Organization. WHO expert committee on gonococcal infections: first report. *Tech Rep Ser* No 262, Geneva; WHO, 1963.
70. World Health Organization. Meeting on *Neisseria* research: first report. *Working document WHO/VDT/RES/Gon. 8.65.*

Br J Vener Dis 1982;**58**:86-91

Higher medical training in venereology*

C B S SCHOFIELD
From Newcastle General Hospital, Newcastle upon Tyne

SUMMARY In a review of the organisation of higher medical training in Britain leading to accreditation for those intending to specialise in venereology within the National Health Service suggestions are made for improving the present system. These include the enrolment of trainees in general medicine as well as in venereology to ensure a broad general experience of medicine. The establishment of an agreed curriculum in venereology for trainees is thought desirable. Improvement in undergraduate education in venereology is necessary if recruitment of trainees to the specialty is to be increased.

The diploma courses in venereology are mainly attended by foreign graduates; the numbers are likely to be reduced by the recent heavy increases in fees. Although adequate practical experience of the tropical venereal diseases cannot be provided, these courses give a good general experience of venereology as ideally practised, much of which is relevant to the conditions under which the overseas trainees will have to work on return to their own countries. Secondment of our own trainees to work in centres in developing countries as an approved part of their training would benefit both them and their hosts.

Introduction

At present there are two quite separate forms of postgraduate training in venereology in Britain. For those who wish to make a career within the National Health Service a three-year period of general professional training, during which the MRCP or equivalent examination is passed, is followed by higher medical training in an approved senior registrar post for up to four years. Courses leading to a diploma in venereology are held in Liverpool and London. Places on these courses are mainly filled by postgraduate students from abroad, some of whom also obtain the diploma of dermatology before returning home to practise dermatovenereology. Currently, the diplomas in venereology are not recognised as a higher qualification for those practising in Britain, although a thorough knowledge of the management of the venereal and other sexually transmitted diseases is necessary to pass these examinations.

Diploma courses

Having considered for some time whether the clinical material available for teaching purposes in England was appropriate for those who return to the tropics to practise, I had the opportunity last year of obtaining some statistics to compare with our own (table I) while on visits abroad.

The figures for England are taken from the annual report of the chief medical officer of the Department of Health and Social Security for the year 1978.[1] The difference in disease incidence between England, India, and Thailand appears to be considerable. We lack the 10-11% of tropical diseases but, apart from these, the table shows a progression from India, where most patients have genital sores of one sort or another and syphilis is still rife, to Thailand, where only a few patients have genital sores and granuloma inguinale is very rare. There, urogenital discharges are the commonest type of disease, with gonorrhoea causing the majority of such infections; these are a legacy from the Vietnam war. In England the venereal diseases are numerically only a minor problem and non-specific genital infections comprise almost half the number of cases of other sexually transmitted diseases. Nevertheless, great strides are being made in the tropics, and standards are

---

*Presidential address delivered to the MSSVD on 24 April 1981

Address for reprints: Dr C B S Schofield, Ward 34, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BF

Accepted for publication 27 October 1981

TABLE I *Percentage distribution of sexually transmitted diseases in three countries*

Diseases	*England*	*India*	*Thailand*
Syphilis	1·1	10·7	1·3
Chancroid		8·4	8·3
Lymphogranuloma venereum		1·3	2·6
Granuloma inguinale		0·7	
Gonorrhoea	14·3	6·5	25·4
Non-specific genital infections	24·1	4·1	11·7
Other sexually transmitted diseases	24·7	32·2	22·1
Other conditions	35·8	36·1	28·6
Grand totals	402 162	452 640	700 216

improving, with more of the other sexually transmitted diseases being diagnosed and treated.

Because the standards vary so much between clinics in each country it is probably fairer to compare the clinical material available for teaching in three centres of excellence: a large clinic attached to a London teaching hospital (personal communication), the Institute of Venereology in Madras,[2] and the Middle Road Hospital in Singapore[3] (table II). If the mean number of cases seen per week at these centres are compared, the main difference between London and the other centres lies in the differential diagnosis of genital sores, London lacking the tropical diseases. Nevertheless, in London and Singapore there are more cases of genital herpes than of primary syphilis, ratios of about 9:1 and 5:1 respectively, while in Madras the ratio is 1:1·3. The London clinic provides the largest caseload of all the other conditions recorded at the three centres, with an excess of non-specific genital infections (although increasing numbers of these cases are now being diagnosed throughout the tropics), and of genital candidosis which remains an uncommon condition at the other centres.

Thus the clinical material available in London is relevant to the practice of postgraduate students when they return home in all aspects save that of the tropical venereal diseases.

TABLE II *Mean number of cases per week seen in three clinics*

Diseases	*London*	*Madras*	*Singapore*
Primary syphilis	1·8	13·4	0·7
Chancroid		20·1	13·3
Lymphogranuloma venereum		5·4	0·5
Granuloma inguinale		6·9	
Genital herpes	17·5	9·7	3·3
Genital warts	19·1	8·4	11·1
Gonorrhoea	96·3	14·7	6·4
Non-specific genital infections	101·2	11·2	6·8
Trichomoniasis	30·6	10·0	2·6
Genital candidosis	41·3	0·4	1·1

In future possibly fewer Asians will enrol for the British courses. Several diploma courses in venereology have been, or are being, set up in India and South-east Asia, which will attract entrants from that part of the world. A course leading to a diploma in dermatology will also soon be established at the Institute of Dermatology in Bangkok.

Two criticisms of the British courses were made when I was abroad. Firstly, the increases in fees for foreign students seeking postgraduate training in Britain were excessive and bound to curtail the numbers able to come from the Third World countries. Secondly, from senior specialists, the facilities in Britain are too sophisticated so that when students returned home they were frustrated by the lack of facilities under which they had to practise, although those who had been on the courses said they had enjoyed them.

While it was generally believed, especially by those advising their governments, that junior specialists should study in their own countries, it was also felt that there was a case for more senior specialists in venereology and microbiology to visit Britain for short sabbaticals to study new and developing techniques and treatments at first hand. Nevertheless, all the specialists with whom I spoke agreed that there was a need for the continued cross-fertilisation of ideas between European and tropical trainees and junior specialists. This, they thought, would be better achieved by Europeans visiting the tropics rather than vice versa, so that they could gain experience in tropical diseases and impart knowledge of the newer techniques and treatments used in Europe.

Training for a career in Britain

Training for career posts in Britain is controlled by the Joint Committee on Higher Medical Training (JCHMT). This is a joint committee of the Royal Colleges of Physicians of Edinburgh, Ireland, and London, and the Royal College of Physicians and Surgeons of Glasgow. Its small secretariat has offices in the London college. The executive consists of representatives of the four colleges, a medical co-ordinator, a post held by the Linacre Fellow of the London college who acts as a link between the executive and the specialist advisory committees (SACs), and representatives of the Association of Clinical Professors of Medicine and of Paediatrics, the Conference of Postgraduate Deans, the Royal College of Pathologists, and the Faculty of Community Medicine. The full committee consists of the executive and a representative from each of the 19 SACs with observers from the various health departments, the Medical Research Council, and a recently appointed consultant. It publishes a training handbook,[4] which includes training programmes for all the specialties and lists of approved senior registrar posts. Previously the full committee met twice a year. More recently the full committee, now with two members from each SAC, meets annually.

The SACs are responsible to the JCHMT for providing visitors to posts whenever educational approval or reapproval is being sought for the training of senior registrars in their specialty. They also make recommendations to the JCHMT on the suitability of trainees for enrolment into higher medical training and on its completion for accreditation as specialists.

At present the SAC on venereology consists of four members elected by the Council of this Society, two by the Royal Colleges of Physicians (to date always members of the Society) and one by the Royal College of Obstetricians and Gynaecologists. The adviser to the chief medical officer of the Department of Health and Social Security is also co-opted. Members of the SAC serve for up to four years and elect their own chairman and secretary, who may serve longer. I have been informed recently that the colleges wish to increase the numbers selected by themselves and to cut down the numbers elected by others, and to limit the time served by chairmen and secretaries.

All the senior registrar posts in venereology have now been visited by the JCHMT over the past five years, and these are being revisited by teams who will recommend to the joint committee whether educational approval can be maintained for another five years, for fewer than five years, or withdrawn. Should approval be withdrawn from a senior registrar post, it is laid down that the incumbent has security of tenure and can continue in training, and having completed higher medical training can be accredited, but the post cannot be used for further training until it has been reapproved.

One of the criteria for educational approval to be given to a post is that the department should be attached to a medical school to ensure a close collaboration with other academic departments in their postgraduate activities. It is laid down that a trainee should be supervised by at least two consultants, each being in the clinic for at least five sessions a week. The workload needs to be of sufficient volume and variety to give the necessary all-round experience. This poses a problem for some clinics where early syphilis is rarely seen. Library facilities within the clinic need to be comprehensive, as far as specialist journals and textbooks are concerned, and should include up-to-date publications in allied specialties. Study leave for trainees should not only be available but should be taken and used for study. Locally it is important that trainees join in the postgraduate activities of other hospital departments.

The visiting teams will also be looking into research facilities, including collaborative work in laboratory medicine, and for evidence that trainees do get experience in emergencies. This may entail their staying in residence when on call. Trainees should rely on their postgraduate deans for advice about their training programmes rather than contacting the SAC direct.

Once in post a senior registrar should enrol for higher medical training. Unless a trainee enrols he cannot later seek accreditation as a specialist. The trainee applies to the secretary of the JCHMT for an enrolment form, who forwards the completed form to the SAC for consideration at their next meeting. If accepted, the form is returned to the JCHMT who notify the trainee.

So far our trainees only enrol with the SAC on venereology, but it is obvious that the four years' higher medical training in venereology alone is excessive; on average they obtain consultant posts within three years. Trainees in the other medical specialties, having obtained the MRCP, still have to enrol with the SAC on general (internal) medicine as well as that of their chosen specialty, and they manage to complete their higher medical training under the two advisory committees within four years.

Before approving an application the SAC looks for evidence that general professional training has been completed and the MRCP or equivalent higher qualification has been obtained. To date if such formal evidence is lacking then information is sought from those who supervised the trainee's general professional training as to its suitability. Ours is the only specialty in which this procedure has been necessary. The SAC can recommend retrospective recognition for periods of time spent in venereology at registrar grade after a higher qualification has been obtained.

The byword of higher medical training is flexibility, each programme being tailored to the individual trainee's needs. Periods spent abroad studying in approved clinics is welcomed, but the agreement of the SAC must be sought beforehand if such periods are to be recognised. If the SAC has doubts about any part of a training programme they can interview the trainee or discuss them with the supervisor.

On completion of their four-year period of higher medical training, or to date on obtaining a consultant post, trainees should apply to the secretary of the JCHMT for an accreditation form. The completed form is returned to him and forwarded to the SAC for its consideration. If the application is approved then a certificate of accreditation is signed by the chairman of the SAC and by the chairman of the JCHMT and forwarded to the trainee.

Is accreditation necessary? In theory, yes. Without it a consultant is not recognised by the JCHMT as a trainer of senior registrars. Lack of accreditation by a consultant could therefore deprive a training post of educational approval. In some instances when the SAC does not believe that the full period of higher medical training has been completed, they may defer accreditation for such periods as they may consider necessary, so called proleptic recognition. In future it is expected that only those possessing a certificate of accreditation will be considered for consultant appointments in the NHS. It is of no use for EEC purposes. To date the EEC only recognises the combined specialty of dermatovenereology, and its possession does not automatically entitle the holder to a consultant post in the National Health Service.

How far has the need for enrolment and accreditation been recognised by our trainees and recently appointed consultants? According to JCHMT records, which may be a little out of date, 56% of our senior registrars in training in 1980 had not yet enrolled and 27% of consultants appointed since 1976 had not applied for accreditation. Five of these are in departments with senior registrars in training.

Recruitment

Unfortunately venereology still does not appear to be very popular among British graduates. Parkhouse and his colleagues[5] noted that only one (0·1%) among 1101 responders had made venereology their first choice of specialty, while according to information on trainees and consultants appointed since 1975 (kindly supplied by the JCHMT) only 59% of our current trainees are British graduates. Lack of interest in our specialty may be due to the fact that few doctors apparently like dealing with basically healthy people or those having social problems[6] and that, in essence, describes our patients. We are, therefore, recruiting from only a small proportion of graduates, few of whom had enough training in the subject as undergraduates to stimulate their interest sufficiently.[7] From information supplied by Professor M W Adler it appears that medical schools providing a total of 10 hours' or less teaching on venereology to undergraduates produced, on average, one trainee per medical school between 1975 and 1980, while those providing over 20 hours of tuition produced an average of two trainees per school. The need for adequate undergraduate training in the specialty appears obvious.

In a survey carried out in 1979 on doctors qualified for between two and 20 years, Hutt and her colleagues[6] noted that while overall 75% of responders had chosen their specialty within five years of qualification, a large proportion of those in venereology were among the 6% of men and 19% of women who had taken 10 years or more. In 1978 the mean age of those appointed as consultant venereologists was 43·8 years.[8] Hutt and her colleagues[6] also noted that for 50% of those in venereology it was their second choice of specialty. This trend may well increase because, according to the JCHMT data, 70% of our trainees in 1978 were making venereology their second choice. While most of these doctors come to terms with the change and add lustre to the specialty, there remain a few who only see a shortage specialty as a means of financial security.

So far there has been some flexibility in the recognition of higher qualifications obtained by our trainees before they have entered higher medical training (even to the extent of accepting training that had not led to a higher qualification), but this cannot be expected to continue for long, because only in venereology is the JCHMT encountering this problem. The proportion of entrants with a higher qualification has risen from 30% of those enrolled during 1975-6 to 89% during 1979-80, but we must not be satisfied until all the entrants for training in venereology are seen to have completed their general professional training, as is expected of the trainees in every other medical specialty.

Career prospects

Venereology is no longer a shortage specialty; the Medical Manpower Division of the DHSS has forecast that for the years 1982-90 the expected number of consultants retiring will be less than five a year.[9] These will be very lean years compared with those of plenty, such as 1978-80, when 24 consultant posts were filled. In addition, according to figures obtained from the JCHMT, there were 36 senior registrar posts in Britain in 1980. Given that there are about 120 consultant posts, the ratio of consultants to senior registrars is about 3·3:1. This is too low, and will ensure that only a minority of trainees will obtain consultant posts on completion of their period of higher medical training. Trainees with the MRCP can be assured of obtaining a consultant post. Of those in training in 1978, 83% of those with the MRCP had become consultants by 1980 compared with 55% of those with the MRCOG and only 35% of those without a recognised higher qualification. Supervisors of trainees lacking the MRCP need to give them every encouragement and opportunity to obtain it, if they are to have a real chance of becoming consultants.

This leads to the conclusion that improvements are needed in recruitment to the specialty, and in our higher medical training generally, to ensure that all senior registrars receive the high standard of training currently provided for only a few, and that a start should be made as soon as is possible. Before any changes can become effective, however, there are two prerequisites. To recruit more British graduates there needs to be a marked increase in the amount of teaching in venereology provided for undergraduates in many medical schools. This need has already been clearly stated.[7 10] Furthermore, the ratio of consultant to senior registrar posts must be increased from approximately 3·3:1 to about 7:1 if all senior registrars are to obtain a consultant post on accreditation. Currently some good potential recruits are deterred, or advised against joining the specialty, because of the uncertainty of obtaining a consultant post.

Some suggestions for improving the present system

Basic to any improvements that may be made in training is the necessity of ensuring that all senior registrar posts are truly supernumary. This will entail improving the staffing levels in those clinics having trainees but will ensure that appointments committees are no longer pressurised to make an appointment at any price, merely to have someone to carry out a service commitment without any hope or expectation of providing adequate training; they can therefore be more selective in their choice of trainees.

Complaints have been made to me from time to time at the JCHMT and appointments committee meetings that some of our trainees and consultants lack that sound knowledge of medicine normally expected of physicians. It does appear that the standing of our specialty among our peers is not as high as would be wished. This, in part, could account for the fact that only 21·7% of our consultants in England and Wales have a distinction award,[11] a proportion significantly below the overall average of 34·3% ($\chi^2_1 = 7\cdot0$; $0\cdot01 > \text{P} > 0\cdot005$). For this reason, among others, I would like to see our trainees enrolled in general (internal) medicine as well as in venereology, especially those whose general professional training did not include experience in acute medicine. Almost all other trainee physicians do this and manage to complete their training in four years, and it has become obvious that most of our trainees in the past have obtained consultant posts within three years. Certainly, if it does not prove possible for them to be enrolled, I think that they should be seconded to work full time in acute medical units for a period of at least 12 months during their training.

The possibility of European trainees and recently appointed specialists spending a period of time studying in clinics in the tropics has been mentioned. This would have to be approved by the SAC. Only in this way will they gain experience in the tropical venereal diseases and other exotic conditions which are seen in Britain from time to time, but whose diagnoses may be missed or mistaken. Very few such diagnoses were made in England during 1978[1] (table III) and very few trainees will have had the chance to see any of these conditions other than yaws. Possibly of greater importance would be the experience of practising, learning, and teaching abroad, and of mixing with peoples of different cultures on their home ground. This would benefit not only the trainees but the health services of the countries concerned. Such visits abroad should be made towards the end of training when the trainees will be well experienced. These visits should be funded centrally and the Government pressed to accept financial responsibility for them.

TABLE III *Tropical venereal diseases and treponematoses reported in England in 1978*

Diseases	*Men*	*Women*
Chancroid	43	4
Lymphogranuloma venereum	26	1
Granuloma inguinale	11	3
Tropical treponematoses	740	400

We recruit trainees with a wide variety and amount of general professional training. Therefore, a curriculum needs to be drawn up to ensure that on accreditation all will have a high standard of knowledge and experience in all aspects of the specialty and are competent to take charge of a clinic on their own. Such a curriculum will need to be both comprehensive and authoritative so that completion of all its components is seen as necessary and useful by both trainees and their supervisors. It should also ensure that trainees know what will be expected of them from the outset and will be able to assess the suitability of the programme mapped out by the supervisor. The academic department of genitourinary medicine at the Middlesex Hospital might consider undertaking the preparation of such a curriculum as part of their educational responsibilities.

The guidelines for training as set out in the Joint Committee's training handbook[4] are naturally sparse. Among obligatory experience I believe it is essential for our trainees to be familiar with the immediate and later management of emergencies, such as pelvic inflammatory disease and Reiter's disease; this may require them to be resident when on call. Opportunity for research is mentioned, but not how to organise research and use statistical methods. Fortunately, most universities run courses on these subjects. Contact tracing, its needs and techniques, is not mentioned although it is vital for disease control. It is hoped that in the future short residential courses on contact tracing will be held for senior registrars at the National Training Centre at Harrogate.

Experience in a number of other specialties is recommended, but minimum periods of such experience could well be laid down in the light of current practice. Nowadays I think that attending a course on psychosexual medicine could replace experience in psychiatry, while attendance at a family planning course is essential to all those who do not have the MRCOG.

In my experience few trainees get much instruction or practice in clinic management or adminstration, and few areas have management courses. The understanding of committee procedures and an understanding of administrators' problems may not appear of importance to trainees but can be vital to their work when they become consultants. The *Special Treatment Clinic: A Design Guide*[12] provides information on recommended staffing levels and the amount of accommodation according to the caseload and is essential in the planning and organisation of a special clinic.

Training programmes are supposed to be tailor-made to suit each senior registrar according to their past experience. With an accepted curriculum in general use it will be possible for the supervisor, trainee, and possibly the postgraduate dean to devise appropriate programmes. I would like to see programmes being sent to the SAC for their approval when the trainee is enrolled for higher medical training and postgraduate deans maintain an informed interest throughout the training period. On appointment, senior registrars should be informed that the first year is probationary, after which their progress will be assessed. At present, such assessment is carried out at only some medical schools, but it should be universal. It should be thorough, and the committee have before it not only a report from the trainee's supervisor but others from all the specialists concerned in the first year's training. The appointment should be terminated if progress is not considered satisfactory, otherwise training will continue. This might be an appropriate time for the trainee to be seconded to an acute medical unit, which will need to have been arranged well in advance by the postgraduate dean, possibly at the time of enrolment.

Finally, how are we to assess the all-round ability of our trainees before they are accredited as specialists? Only a few venereologists who passed the old Edinburgh MRCP examination, specialising in venereology, have provided evidence of having completed higher training in medicine and venereology. Should our trainees sit a test based on the curriculum? I have spoken to several of those concerned with the diploma courses and they are generally agreed that, as currently organised, it would not be possible for all the trainees in Britain to sit these examinations, although an examination organised nationally might prove feasible. But do we need, and can we afford, yet another postgraduate diploma or certificate?

Discounting an examination, I would suggest that the SAC should receive, with the application for accreditation, a detailed report on the whole of the training period, which should provide sufficient evidence that the programme agreed upon at enrolment has been completed satisfactorily. A certificate of accreditation should not be given merely on the basis of a trainee having occupied a senior registrar's post for a set period of years but on the basis of having completed a satisfactory training programme.

I hope that my thoughts and suggestions for changes in higher medical training in our specialty will be considered as constructive and, if not generally accepted, will lead to others devising improvements which will be to the benefit of the specialty, to the doctors working in it, and to our patients.

I am indebted to Dr Dharem Pal, adviser in STDs to the Government of India, and to Dr Kalya Suthisomboon, director of the VD Control Division and Teaching Centre of the Department of CDC, Ministry of Health, Thailand, for the statistics pertaining to India and Thailand respectively (table I).

References

1. Department of Health and Social Security. Sexually Transmitted Diseases. *On the State of the Public Health: The Annual Report of the Chief Medical Officer of the Department of Health and Social Security for the Year 1978*. London: HMSO, 1979:60-5.
2. Institute of Venereology. *Annual Report, 1978*. Madras: Madras Medical College, 1979.
3. The Medical Superintendent. *Annual Report, 1979*. Singapore: Middle Road Hospital, 1980.
4. The Joint Committee on Higher Medical Training. *Training Handbook and Third Report*. London: Royal College of Physicians, 1980.
5. Parkhouse J, Parkhouse HF, Hambleton BA, Bullock N. Careers of doctors qualifying in Britain in 1974: A follow-up in 1979. *Health Trends* 1981;13:51-6.
6. Hutt R, Parsons D, Pearson R. The timing and reasons for doctors' career decisions. *Health Trends* 1981;13:17-20.
7. Anonymous. Undergraduate medical education in genitourinary medicine. *Br Med J* 1981;283:572.
8. Medical Manpower Division, Department of Health and Social Security. Tables on consultant vacancies and other topics. England and Wales, year ending 30.9.78. *Medical Manpower Documents* 1979; table 14.
9. Medical Manpower Division, Department of Health and Social Security. Medical staffing and prospects in the NHS in England and Wales, 1980. *Health Trends* 1981;13:57-60.
10. Adler MW, Willcox RR. Teaching of genitourinary medicine (venereology) to undergraduate medical students in Britain. *Br J Vener Dis* 1981;57:170-3.
11. Department of Health and Social Security. Distinction award holders in England and Wales. *On the State of the Public Health. The Annual Report of the Chief Medical Officer of the Department of Health and Social Security for the Year 1979*. London: HMSO, 1980:110-1.
12. *Special Treatment Clinic: A Design Guide*. London: Department of Health and Social Security, 1974.

UNIVERSITY COLLEGE LIBRARY

Br J Vener Dis 1982;**58**:92-5

Role played by Italy in the history of syphilis

M A WAUGH
From the Department of Genitourinary Medicine, General Infirmary, Leeds

Introduction

Despite dissenting views on the origin of the well-recorded outbreak of syphilis in the Italian peninsula towards the end of the fifteenth century, there is no disputing the importance of Italian culture and medicine in the development of the history of syphilis. In the earlier part of this century most of the detailed work on the subject was recorded by medical historians of the German School. Bloch[1] was the chief supporter of the theory of the Columbian origin of syphilis. Sudhoff,[2] while making no dogmatic assertion about the origin, was the principal collator of early sources in European literature, showing the intimate connection between the contemporary life of Italy, Spain, and Germany.

Origin of syphilis in Europe

The followers of the Columbian theory of the origin of syphilis do not consider that syphilis existed in western Europe before 1493, its advent coinciding with the return of Columbus, a Genoan, and his crew after the discovery of the New World.

This view is opposed, however, by the followers of the unitarian concept of the treponematoses such as Hudson.[3] This concept emphasises the evolutionary relationship of yaws, pinta, endemic syphilis, and sporadic syphilis, regarding them all as varieties or syndromes of one disease caused by one parasite, the *Treponema pallidum*. This concept considers sub-Saharan Africa to be the area for the origin of treponematosis.

The unitarian theory explains the epidemic in Europe at the end of the fifteenth century in two alternative ways. Firstly, the disease could have existed in some mild form in Europe for centuries, the organism suddenly developing a new and deadly virulence.[4] Secondly, the epidemic may have stemmed from an alteration in the organism of yaws, which had been brought back to Spain and Portugal by travellers.

Syphilis is usually said to have first appeared at the Siege of Naples in 1494-95, but Caspare Torella,[5] a Spanish physician to Pope Alexander VI, stated that it was known in Avignon in 1493. There was a concept of a disease like syphilis which was distinct from leprosy and chronic skin disease in Provence for a considerable period, perhaps explaining the term 'mal franzoso'. Some authors[2] have thought that scabies grossa, mentioned in Italy as early as the thirteenth century and treated with mercury, could have been syphilis.

There is even a problem in dating the Siege of Naples. Charles VIII of France claimed the throne of Naples from Ferdinand II. At that time the Gregorian calendar was used in Italy, and Naples was besieged by the French from 21 February 1495. According to the French calendar the date was 1494, as the year began at Easter (19 April). Gabriele Fallopius[6] (1523-62) related in 1563 that the harlots were driven out to the welcoming arms of the besiegers and before long the French army had to withdraw, being stricken with the Neapolitan disease, from whence it spread all over Europe. Like so much that followed in the history of venereal diseases, it is often difficult to unravel what in fact did happen; different authors interpret differently and often add to and even exaggerate the pattern of events.

Early writers on syphilis in Italy

In the last few years of the fifteenth century several writers in Italy described syphilis. Niccolo Leoniceno of Vicenza[7] (1428-1524), professor of medicine at Ferrara, wrote a pamphlet in conjunction with the humanist, Aldus Manutius, of Venice in June 1497, in which he described the disease and included its differential diagnosis. Leoniceno stated that there had been accounts of a similar disease as early as Hippocrates, in whose *Aphorisms* (book 3, chapter XX) it is mentioned, 'that in the summer ulceration of the mouth, skin eruptions, and mortification of the privy parts occurs'.

Torella wrote two important works on syphilis in November 1497 and between 1499 and 1500. In the first,[5] he called the new disease 'pudendagra' and gave a description of how a patient caught the

Address for reprints: Dr M A Waugh, Department of Genitourinary Medicine, General Infirmary, Great George Street, Leeds LS1 3EX

Accepted for publication 25 August 1981

affliction in the month of August. It started with an indurated ulcer on the penis and enlargement of the inguinal glands, followed later by pains in the head, shoulders, breast bone, arms, legs, and ribs and then by multiple pustules on the head, face, and chest 10 days later. Thus, he was the first to describe the chancre, related lymphadenopathy, osteoscopic pains, and the secondary eruption. It may be that Torella's subject was the notorious Cesare Borgia (1476-1507), the son of Alexander VI, who was made a cardinal at the age of 15 in 1492, which would have made him 20 at the time of Torella's description. Torella described five cases; he alluded to divine wrath as the cause and relied on mercurial ointment for treatment. In his second work[8] (1499-1500) he described how healthy infants could be infected from breast sores of a syphilitic wet nurse and how one nurse could contaminate a great number of suckling babes.

Corradino Gilino,[9] probably a pupil of Leoniceno at Ferrara, recommended (1497) more use of mercurial inunction, adding sublimate of mercury to mercury in metallic form, although later he retracted this idea, restricting sublimate to washes and fomentations. He advised against too much blood letting, although he favoured the rather gruesome therapy of applications of a red-hot iron to the head in the region of the coronal sulcus, thinking thereby to influence the pituitary, the source of phlegm.

Natale Montesaûro of Verona[10] (1498) wrote his *Tractate* as much to attack Leoniceno as to describe syphilis. He stated he had long been familiar with the disease, and he probably gave a better description of pains in the long bones than Torella. Unlike the latter, he discounted mercury but stated that he had seen cases of the disease without any primary infection of the genitals.

The last of the medical writers from Italy in the short period before the end of the fifteenth century was Antonio Scanaroli of Modena[11] (1480-1517). He supported Leoniceno, but made the peculiar statement that, 'the disease is not passed only through intercourse, but could arise de novo'. He alluded to it occurring in virgins and old men who had no sex life.

The Guicciardini were an important Florentine family and a member of it, Francesco Guicciardini,[12] who wrote a history of his times from 1494 to 1532, stated that, 'the physicians understood nothing of the treatment of this disease, and, instead of the appropriate medicines, they frequently gave remedies which were the opposite and capable rather of irritating the disease'.

Sixteenth century descriptions in Italy

The writings of Antonio Benivenio (1502), a Florentine physician, were published (1507) after his death.[13] They are remarkable for detailed descriptions of different forms of cutaneous lesions of the disease.

John of Vigo (1514) wrote his great and influential surgical treatise in Rome, in which he included two chapters on the French disease.[14] Of importance in the clinical description of the disease is the mention of ocular manifestations. Like most others of the time he advocated mercury as treatment in one form or another.

FRACASTORO

There are several other Italian writers on venereal disease in the first part of the sixteenth century, but none of such importance as Girolamo Fracastoro (1478-1553), a native of Verona, practising in physic. In 1525 Fracastoro had written a poem in Latin hexameters on syphilis, dedicating it to his patron, Cardinal Pietro Bembo. He alluded to mercury from Mount Etna but after some criticism was persuaded to change the poem somewhat to exclude mercury, then out of favour, and include references to guaiacum, which was by then in vogue for the treatment of morbus Gallicus. The work, *Syphilis sive Morbus Gallicus,*[15] was published in a small quarto of 36 pages in August 1530 and consists of three books. In the first the astrological causes of the new disease are mentioned. Then, two-thirds of the way through, a clinical description of the disease is given. It ends in the classical style of Virgil. The second book again eulogises Bembo. Syphilus makes his entry in line 288 of the third book of 419 lines. The shepherd, Syphilus (though some would suggest 'syphilus' means pig-lover, 'sus' being Homeric Greek and Latin for pig), watched over the flocks of King Alcithous and one day reproached Apollo for parching the trees and draining the springs in such a manner that his flock was dying of want of shade and lack of water. He swore he would in future sacrifice to his king and not to the sun. Outraged, Apollo inflicted a disease so far unknown, which afflicted the whole population, including the king. On the advice of the nymph, Ammerice, the inhabitants offered sacrifices to Juno and Tellus and the latter produced the guaiacum tree which cured the disease. To make absolutely sure, the priests offered Syphilus as a sacrifice to Apollo, but Apollo accepted a bullock instead. The importance of this poem was enormous, not only in giving the disease a name but in being translated into most European languages.

America had been officially recognised in atlases of 1518 and 1524. Guaiacum was indigenous to the West Indies and its importation was a monopoly of the House of Fugger of Augsburg. It had been imported since 1508, its popularity being assured by Ulrich von Hutten[16] in his work (1519) *De Morbi*

Gallicum Curatione per Adminstrationem ligni Guaiaci. Verona lies at the start of the easiest route over the Alps, by way of the Brenner Pass, and there would have been regular commerce between Verona and southern Germany.

The name syphilis was not used in English until 1686, when the dramatist Nahum Tate[17] translated Fracastoro's work: the first mention in English medical writing was by Daniel Turner[18] (1717).

AFTER FRACASTORO

About the same time as Fracastoro, Niccolo Massa of Venice (1537) wrote a work in five parts on morbus Gallicus.[19] He admitted that coitus was the usual cause of the disease, but not the only cause. He described congenital syphilis. Previously he had thought the disease had been contracted by infants who had taken the mother's breast milk, but he was able to show that it had occurred in three children who had not suckled and must thus have been passed in infected blood. He also described the necropsy appearances of syphilis. He should be noted for an early method of prophylaxis, washing the genitals of both men and women after intercourse with a mixture of cold white wine and vinegar. He recommended guaiacum, sarsaparilla, and china root but mercury only with reserve.

Antonio Brassavolus (1551) of Ferrara is noted for dividing morbus Gallicus into 234 types.[20] He did, however, mention deep kissing (oscula cum vibratione et conflicto linguarum) as a cause of chancre of the mouth and was among the first to describe anal chancre. He considered gonorrhoea, however, to be part of the great disease of verola (syphilis).

Gabriele Fallopius, a pupil of Vesalius, is not only celebrated for describing the anatomical parts named after him but also for an important posthumous work on syphilis.[6] He was an influential exponent of the Columbian theory. He had some interesting ideas on the aetiology of syphilis, noting that men who were circumcised or had the glans exposed were less likely to contract the disease than those who had a long prepuce which easily became injured. He described the course of the disease, and considered an indurated chancre to be pathognomonic. He noticed that syphilis caused alteration to the throat (with roughness of the voice), nocturnal pains, and lesions of the bones and gummata. Although he used mercury by inunction he preferred guaiacum, China root, and sarsaparilla. He gave a good description of phimosis and its cure and described condylomata lata. He is best remembered as a very early protagonist of the condom, advising those in need to carry one at all times. Like Massa, he also believed in preventive antiseptic measures and described various remedies.

Eighteenth century advances

The Italian School waned in the seventeenth century. Giovanni Maria Lancisi of Rome (1654-1720), however, was one of its most brilliant lights, leaving science enriched by his many discoveries. Venereology has been especially helped by the second of his two great works, *De Motu Cordis et Aneurysmatibus*[21] published in 1728 after his death. In this work he described Gallic (syphilitic) aneurysm, 'it being known not only by the impure intercourse that has gone before and signs of venereal disease that have already spread to other parts besides, but above all it is identified by the manner in which a definite place is attacked by the aneurysm.'

Perhaps the founder of modern pathology can be considered to be Giovanni Battista Morgagni (1682-1771). He was a pupil of Valsalva and published his great book[22] *De Sedibus et Causis Morborum* after 60 years of observation and unhurried thought at the age of 79 in 1761, living to see several reprints and not dying until his ninetieth year. The work of Morgagni on aneurysms is probably better than that of Lancisi. He noted that sudden death resulted from rupture of aneurysms and that they commonly occurred in men whose occupations were susceptible to syphilis, such as guides, postillions, and horseback riders. He recorded the occurrence of endocarditis after virulent gonorrhoea but unfortunately made no distinction between gonorrhoea and syphilis, considering lues venerea to be all one disease. He also made the common mistake of the times of attributing to syphilis all the lesions occurring in the body of a sufferer from that disease, stating that 'the lungs, aorta, kidneys and their appendages are found injured in those who have suffered considerably and for a long time with lues venerea.' It is of interest to the medical historian that James Boswell, while travelling in Italy in 1765, consulted Morgagni (then 83) at Padua. The aged physician told him to lead a sober life, stop using the syringe, and to let nature heal itself.[23] Boswell unfortunately did not heed the warning.

Hypodermic syringe and nineteenth century advances

Syringes have been known in medicine since the earliest times. For purposes of anatomical demonstration Galen injected the cerebral vessels. Leonardo da Vinci injected the cerebral ventricles with wax, and Harvey used injections of dye. From time immemorial syringes have been used for urethral, vaginal, and rectal irrigation and for the probing and introduction of fluids into sinuses and fistulas.

Hypodermic use, however, does not seem to have come about until the nineteenth century. Alexander Wood (1855) of Edinburgh was among the first to use the syringe for this purpose. Among the first in venereology was Angelo Scarenzio of Pavia, who published a work on the treatment of constitutional syphilis with subcutaneous injections of mercury into the thigh in 1864. There was an immediate improved response in the patients,[24 25] but unfortunately abscesses often occurred at the sites of injection. Carlo Ambrosoli of Milan[26] and Berkeley Hill[27] in London published similar results. Hebra in Vienna had earlier tried subcutaneous injections but had given them up when too many side effects occurred.

Notable experimental work was carried out by Pietro Pellizzari (1823-92) of Florence, who proved the possibility that syphilis could be transmitted by blood transfusion.[28 29] In February 1862 he inoculated blood from the cephalic vein of a women with secondary syphilis on to the arms of three young volunteer doctors. One of them (Bargioni) developed a chancre at the point of inoculation on the left forearm after 22 days as well as axillary lymphadenopathy. It was mentioned that mercury treatment followed, but the final fate of Dr Bargioni is unknown.

History tends to repeat itself. Giuseppe Profeta (1840-1911) wrote on congenital syphilis.[30] Others have labelled his findings as Profeta's law—that is, the healthy child of a syphilitic mother is immune to syphilis. In fact Profeta was supposed to have implied that the healthy child of a syphilitic mother could be suckled by her or by a syphilitic nurse with impunity. In the light of advances in immunology, which were not to come until the twentieth century, the law rapidly became outdated.[31] Profeta had not realised that an apparently healthy child could have latent congenital syphilis. The syphilitic infant is often asymptomatic at the time of birth and for the ensuing few weeks. By the late nineteenth century advances into research and the treatment of syphilis no longer seem to have come from Italy; the German-speaking countries then made the greatest contribution.

References

1. Bloch I. *Der Ursprung der Syphilis.* 1st ed. Jena; Gustav Fischer, 1901.
2. Sudhoff K. *The Earliest Printed Literature on Syphilis.* Florence: R Lier, 1925.
3. Hudson EH. *Treponematosis.* Oxford: Oxford University Press, 1946.
4. Hackett CJ. On the origin of the human treponematoses. *Bull WHO* 1963;29:7-41.
5. Torella C. *Tractatus de Pudendagra seu Morbo Gallico.* Rome, 1497.
6. Fallopius G. *De Morbo Gallico.* Padua: L Gryphium, 1563.
7. Leoniceno N. *Libellus de Epidemia quam vulgo Morbum Gallicum vocant.* Venice: Aldi Manutii, 1497.
8. Torella C. *Dialogus de Dolore cum Tractatu de Ulceribus in Pudendagra evenire solitis.* Rome, 1499-1500.
9. Gilino C. *De Morbo quem Gallicum noncupant.* Ferrara, 1497.
10. Montesauro N. *De Dispositionibus quas Vulgares Mal Franzoso appellant.* Verona, 1498.
11. Scanaroli A. *Disputatio de Morbo Gallico.* Bologna, 1498.
12. Guicciardini F. *La Historia d'Italia Vinegia.* Presso Giorgio Angelieri, 1583.
13. Benivenio A. *De Morbo quem vulgo Gallicum vocant.* Florence, 1507.
14. Vigo J. *Practiosa Copiosa in Arte Chirurgica.* Rome, 1514.
15. Fracastoro G. *Syphilis sive Morbus Gallicus.* Verona: S Nicolini da Sabbio, 1530.
16. Von Hutten, U. *Von der Wunderlichen Arzenei, des Harzes, Guaiacum genannt und Wieman die Franzosen beilen soll.* Strasburg, 1519.
17. Tate N. *Syphilis or a Poetical History of the French Disease.* London: J Tonson, 1686.
18. Turner D. *Syphilis. A Practical Dissertation on the Venereal Disease.* London, 1717.
19. Massa N. *Liber de Morbo Gallico.* Venice, 1532.
20. Brassavolus AM. *Ferrarensis, de Morbo Gallico.* Venice, 1551.
21. Lancisi GM. *De Motu Cordis et Aneurysmatibus.* Rome: Salvioni, 1728.
22. Morgagni GB. *De Sedibus et Causis Morborum.* Venice: Remondiniana, 1761.
23. Ober WB. *Boswell's Clap and Other Essays.* Carbondale and Edwardsville: Southern Illinois University Press, 1979;11.
24. Scarenzio A. Subcutaneous injections of a preparation of mercury against constitutional syphilis. *Ann Universali de Med Milano* 1864;189:602.
25. Scarenzio A. Hypodermic injection of calomel in syphilis. *Br Med J* 1865;ii:60.
26. Ambrosoli C. Sul modo di curare in sifilide constituzionale colle iniezioni sottocutane di un preparato di mercurio. *Giornale Ital delle Mal Veneree* 1866;1:97-116.
27. Hill B. Subcutaneous injection of mercury in constitutional syphilis. *Lancet* 1866;i:498.
28. Pellizzari P. *Della Transmissione delle Sifilide Mediante l'Inoculazione del Sangue. Lo Sperimentale.* Florence, 1862.
29. Pellizzari P. Successful inoculation of syphilitic blood. *Br Med J* 1862;i:495-6.
30. Profeta G. Sulla sifilide per allattamento. *Sperimentale* 1865;**15**:328-38,339-418.
31. Nabarro D. *Congenital Syphilis.* London: Arnold, 1954:66.

Br J Vener Dis 1982;**58**:96-100

Inhibition of *Neisseria gonorrhoeae* isolates by Martin-Lewis medium

Epidemiology, susceptibility profile, and plasmid analysis

MARCIA A MILLER, PATRICK ANDERSON, JOHN W PARKER, AND H HUGH ROHRER

From the Department of Basic Sciences, Peoria School of Medicine, University of Illinois, and the Peoria City/County Health Department, Peoria, Illinois, USA

SUMMARY A study was undertaken on the premise that if an increase of 1 μg/ml in the concentration of vancomycin in Martin-Lewis medium (MLM) could improve inhibition of Gram-positive cocci, then a concomitant increase in the inhibition of gonococci could also occur. Isolates of *Neisseria gonorrhoeae* that failed to grow on MLM accounted for 18·5% of all positive culture results for gonorrhoea. The incidence of isolates susceptible to vancomycin was 14% and of those susceptible to trimethoprim 2·4%; one isolate was susceptible to both vancomycin and trimethoprim. The antibiotic-susceptible isolates were more frequently isolated from asymptomatic white men. Plasmid analysis showed that the 2·4-megadalton cryptic plasmid was absent in the vancomycin-susceptible isolates. The large proportion of isolates failing to grow on selective MLM has important clinical implications for the diagnosis of gonorrhoea.

Introduction

The importance of cultural methods for the diagnosis of gonorrhoea is well documented and numerous modifications directed towards improving cultural yields have been published.[1] A modification of the Thayer-Martin medium[2] was introduced by Martin and Lewis in 1977,[3] in which the vancomycin concentration was increased from 3 μg/ml to 4 μg/ml, and the antifungal agent, nystatin, was replaced by anisomycin. The basis for this modification was to minimise overgrowth of cultures by yeasts and Gram-positive organisms such as staphylococci.

The new Martin-Lewis medium became the medium of choice in many sexually transmitted disease (STD) clinics and other laboratories processing cultures for gonorrhoea before it was subjected to sufficient clinical trials.[4] This caused concern because of the increase in concentration of vancomycin and reports of vancomycin-susceptible strains of *Neisseria gonorrhoeae.*[5-10]

This paper reports on the inhibitory qualities of the Martin-Lewis formulation, the susceptibility profiles of isolates failing to grow on this medium, and a plasmid analysis of these isolates.

Address for reprints: Dr M A Miller, Peoria School of Medicine, P O Box 1649, Peoria, Illinois 61656, USA

Accepted for publication 13 October 1981

Materials and methods

CULTURAL PROCEDURES

The specimens were obtained from consecutive outpatients examined at the STD clinic, Peoria City/County Health Department, Peoria, Illinois, between 12 February and 22 March 1979. Specimens were collected from the urethra of men and the cervix of women. Men with a history or evidence of urethral discharge or dysuria or both were classed as symptomatic. Patients with no history or evidence of discharge and dysuria were classed as asymptomatic.

Martin-Lewis medium (MLM) containing 4 μg/ml vancomycin, 7·5 μg/ml colistin, 20 μg/ml anisomycin, and 5 μg/ml trimethoprim lactate was supplied in a single lot by Scott Laboratories, Fiskeville, RI, USA. Quality control was performed by the manufacturer. Chocolate agar plates (CAP) with 1% haemoglobin and 1% supplement C (Difco, Detroit, Michigan) were made daily.

Specimen swabs were immediately placed into a labelled tube of 5·0 ml brain heart infusion broth (BHI, Difco, Detroit) and processed in the laboratory within 15-30 minutes of receipt. The swab and broth were vortexed for 20 seconds and the swab removed. Fresh sterile swabs (Dacron, No A5005-1, American Scientific Products) were immersed in the BHI for 20 seconds and an 'N' configuration streaked on to MLM and CAP; a fresh swab was used to streak each medium. The 'N' configuration was cross-streaked with a sterile loop and incubated at 35°C in 6% CO_2 with readings at 48 and 72 hours. Gram-negative oxidase-positive diplococci were identified by a carbohydrate utilisation test using the medium described by Flynn and Waitkins,[11] with the addition of 3·5 µg/ml cocarboxylase (Sigma, St Louis, Missouri). Heat-fixed suspensions of vancomycin-susceptible isolates were also treated with antigonococcal globulin conjugated with fluorescein isothiocyanate for identification purposes.

SUSCEPTIBILITY TESTING

Minimum inhibitory concentrations (MIC) were determined in Mueller-Hinton broth with 1% supplement C (Difco, Detroit) and by an agar dilution method using GC agar base with 1% supplement C and 1% haemoglobin.[12] An inoculum of 5×10^5 organisms was used. Susceptibility testing for trimethoprim was performed in trypticase soy media (Difco, Detroit) supplemented with 1% Isovitalex. Cultures were incubated at 35°C in 6% CO_2 and read after 24, 48, and 72 hours.

Vancomycin hydrochloride (Vancocin® HCl) was supplied as a sterile dry powder in 500-mg vials (Eli Lilly and Co, Indianapolis). Trimethoprim lactate (Burroughs Wellcome Co, Research Triangle Park, North Carolina), spectinomycin sulphate (Trobicin, Upjohn Company, Kalamazoo, Michigan), cefoxitin (Merck, Sharp and Dohme, Rahway, NJ), and commercial preparations of penicillin G and tetracycline hydrochloride (Pfizer Inc, New York) were used.

CULTURE AND DNA EXTRACTION

N gonorrhoeae isolates to be examined for plasmids were grown in 500 ml trypticase soy broth (TSB) containing 0·01 mol/l $MgSO_4$ and 0·01 mol/l $NaHCO_3$, pH 7·2, for 8-12 hours at 35°C. *E coli* strain V517, which contained plasmids of known specific molecular weights, was supplied by Dr Ester M Lederberg (Plasmid Reference Center, Stanford University School of Medicine, Stanford, California). Cultures for extraction purposes were grown in TSB and harvested after 12 hours' incubation at 35°C. The DNA extraction procedure included the Guerry *et al* [13] method for lysing cells followed by the ribonuclease, deproteinisation, and precipitation steps described by Meyers *et al.*[14]

AGAROSE GEL ELECTROPHORESIS

The 0·7% or 1·0% agarose (w/v) gel was electrophoresed at 30 mA at room temperature until the dye was 10 mm from the bottom of the gel. The gel was then placed in a solution of ethidium bromide (0·05 µg/ml) and stained for 15 min.[15 16] Gels were photographed on a black background under ultraviolet light (375 nm) for 15-20 min using a 35 mm camera with Wratten No 22 orange filter.

STATISTICAL ANALYSIS

Results were evaluated for statistical significance by Student's *t* test.

Results

CLINICAL AND CULTURE RESULTS

A total of 346 men were examined and gonococci isolated from 58 (16·8%); 105 women were examined and gonococci isolated from 23 (21·4%). Of the positive cultures, 10 of 58 (17·2%) from men and five of 23 (21·7%) from women failed to grow on MLM after 96 hours of incubation at 35°C in 6% CO_2. During repetitive subculturing, two isolates were lost from a white symptomatic man and a white asymptomatic woman. Therefore, 13 of 79 (16·5%) positive cultures that failed to grow on MLM remained for further testing.

Details of the patients from whom these 13 strains were isolated are shown in table I. The proportion of isolates failing to grow on selective MLM in men (17·2%) and women (21·7%) was comparable. The frequency of recovery of such isolates was three times greater in white than in non-white patients and the individual was more likely (2½ times) to be an asymptomatic white man.

TABLE I *Clinical features of 13 patients infected with gonococci which failed to grow on selective (MLM) media.*

	Race			
	White		*Non-white**	
Sex	*Symptomatic*	*Asymptomatic*	*Symptomatic*	*Asymptomatic*
Male	2	5	2	0
Female	2	1	0	1

* All were black

SUSCEPTIBILITY PROFILES

Vancomycin MICs of isolates failing to grow on the selective MLM media were <2 µg/ml for nine isolates, >2 but <3 µg/ml for two, and >8 µg/ml for the remaining two strains. The rate of vancomycin susceptibility (MIC <3 µg/ml) was 14% of all positive cultures for *N gonorrhoeae*. One isolate which failed to grow on the selective medium was resistant to vancomycin but susceptible to trimethoprim. Another isolate was inhibited by both vancomycin and trimethoprim. Susceptibility to trimethoprim was 2·4% of the total positive cultures.

All of the 13 isolates failing to grow on MLM were susceptible to <1 μg/ml penicillin (range 0·011-0·78 μg/ml). Susceptibility was noted to tetracycline (range 0·011-1·56 μg/ml), spectinomycin (range 0·045-12·5 μg/ml), and cefoxitin (range 0·045-1·56 μg/ml). Thirteen control isolates, matched for the patients' age, sex, race, and day and week of collection were examined. The mean MIC for each antibiotic for the vancomycin-sensitive and control organisms is shown in table II. The data between the groups were compared by a standard Student *t* test. There was a significant difference (P>0·997) in the mean vancomycin MIC between the two groups of isolates but no significant difference in the mean MICs for the other antibiotics.

With a high inoculum (10^6 cfu) four of 13 isolates, originally failing to grow on MLM, overcame the inhibition of vancomycin on subculture. Only two of the four did so, however, when a 10^3 inoculum was used. The strains which grew on vancomycin-containing plates with both inocula had MICs of 12·5 and 25 μg/ml. The other two isolates had MICs of 2·5 μg/ml. All isolates with MICs <2·0 μg/ml failed to grow on vancomycin-containing media regardless of the inoculum size. Neither of the two trimethoprim-sensitive isolates grew when subcultured to trimethoprim-containing media with inocula of either 10^3 or 10^6 cfu.

PLASMID ANALYSIS

Six isolates which failed to grow on MLM were analysed for their plasmid content and compared with six isolates with vancomycin MICs >8 μg/ml. The gel patterns of two isolates with vancomycin MICs >8 μg/ml are shown in the figure (lanes 2 and 3). Compared with standard molecular-weight plasmids in lane 1, a single 2·6-megadalton plasmid was observed in both isolates. Lane 4 contains an isolate with a vancomycin MIC of 2·5 μg/ml which overcame the inhibition of vancomycin only with an inoculum of 10^6 cfu. A 2·6-megadalton plasmid is evident.

Lanes 5-7 show three vancomycin-susceptible isolates, with lane 7 representing the isolate susceptible to both vancomycin and trimethoprim. The 2·6-megadalton plasmid is absent in each of these three isolates. In preparations (not shown) of two additional isolates susceptible to vancomycin, the 2·6 megadalton plasmid was also absent. All control isolates tested (vancomycin MIC >8 μg/ml) contained the 2·6-megadalton plasmid as did the two isolates which initially failed to grow on MLM but were later found to be vancomycin-resistant. No 25-megadalton plasmid was observed in any of the isolates studied.

Discussion

Isolates of *N gonorrhoeae* that failed to grow on MLM accounted for 18·5% of organisms isolated over a six-week period. Of the 15 isolates recovered, 13 were further evaluated; 11 (14%) were susceptible to <3 μg/ml vancomycin. Susceptibility to trimethoprim was observed in two (2.4%) isolates. While approximately 10 reports on vancomycin susceptibility have been published since 1969, fewer have cited susceptibility to trimethoprim.[5,17–19] Taylor and Phillips[5] reported that only 1% of their isolates had MICs <4 μg/ml. Our data illustrate a similar small proportion of isolates inhibited by concentrations of trimethoprim present in MLM. This would indicate that trimethoprim should not present any undue problem of inhibition of strains when incorporated into selective media.

In other studies the incidence of susceptibility to vancomycin has been around 3-4%[5–7] and 8-12%.[8–10] The 14% incidence of vancomycin susceptibility in this study falls in the latter group. Windall *et al* have reported a 30% incidence of gonococcal cultures failing to grow on modified Thayer-Martin media.[20]

There is little published information on the relationship between vancomycin-susceptible strains and MICs to other antibiotics. Antibiotic susceptibility is a mark of virulence for some gonococci.[21,22] Marked susceptibility to penicillin (MIC⩽0·015 μg/ml) has been found in strains from disseminated gonococcal infection (DGI)[21,23] and from patients with

TABLE II *Mean MICs of six antibiotics for test and control organisms*

	Mean MIC (μg/ml) ± SD of following antibiotics:					
*Organisms**	*VAN*	*TMP*	*PEN*	*TET*	*SPC*	*CFX*
Test	3·43 ± 7·31	71·42±40·12	0·46±0·55	0·76±0·65	5·23±5·17	0·37±0·39
Control	30·28±26·25	61·53±33·25	0·42±0·31	0·76±0·49	6·55±5·09	0·36±0·38

VAN = vancomycin; TMP = trimethoprim; PEN = penicillin; TET = tetracycline; SPC = spectinomycin; CFX = cefoxitin; SD = standard deviation.

* Three MIC values were estimated for each of 13 isolates in both test and control groups.

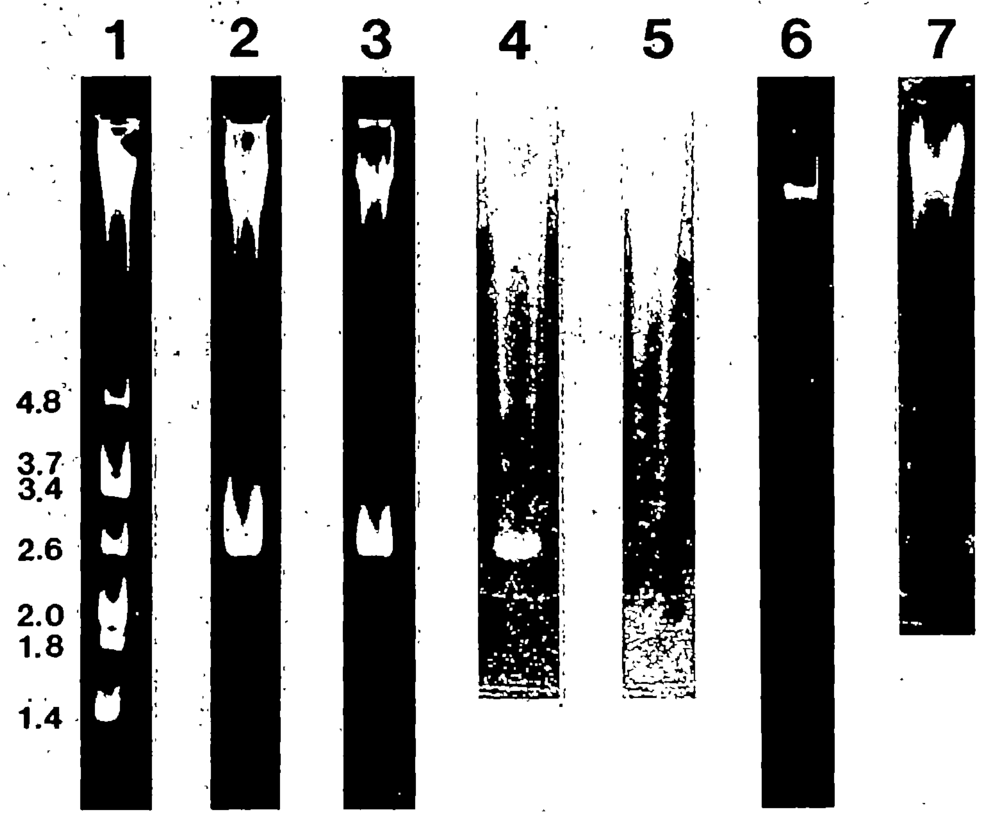


FIGURE *Agarose gel electrophoresis of plasmid DNA. Lane 1 contains E coli strain V517 with standard molecular-weight plasmids. Lanes 2 and 3 show vancomycin-resistant isolates (MIC>4 μg/ml), which contain a 2·6-megadalton plasmid. Lane 4 contains a vancomycin-susceptible isolate that was able to grow in the presence of vancomycin with a large (10^6 cfu) inoculum and had a vancomycin MIC of 2·5 μg/ml. Isolates with vancomycin MICs <2·0 μg/ml (lanes 5-7) did not contain a 2·6-megadalton plasmid.*

asymptomatic urethral infections. The penicillin MICs for our vancomycin-susceptible isolates were comparable to those of Thompson *et al*[24] for non-DGI isolates, with 72% of all isolates having penicillin MICs ≥0·125 μg/ml. Our findings for susceptibility to tetracycline were, however, similar to those for DGI isolates[24] with 60% having MICs of 0·125-0·250 μg/ml. These MICs of tetracycline are equivalent to those reported for gonococcal isolates examined in the National Gonorrhea Therapy Monitoring Study (NTMS)[25] from 1972 to 1977. MICs for spectinomycin and cefoxitin were comparable to NTMS results.[25]

Many drug resistant markers in bacteria are located on plasmids. Agarose gel electrophoresis of vancomycin-susceptible isolates showed the absence of the indigenous 2·6-megadalton plasmid when the vancomycin MIC was <2·0 μg/ml. While this plasmid does not universally appear in gonococcal isolates, reported rates range between 83-100%.[26-28] Sparling *et al*[29] have attributed vancomycin susceptibility to envelope (*env*) mutations. Although the outer membrane of these isolates has not been analysed, recent data from Miller *et al*[12] suggest that the cytoplasmic membrane may also be an important determinant of antibiotic susceptibility or resistance in *N gonorrhoeae*. While the loci for *env* mutations are purported to be chromosomally located,[30] further genetic analysis of these vancomycin-susceptible isolates are warranted in view of our plasmid findings.

The large proportion of isolates failing to grow on selective MLM has clinical implications for the diagnosis of gonorrhoea. This report is to alert both physicians and laboratory personnel to yet another basis for false-negative culture results. A change in antibiotic composition of gonococcal selective media may be warranted. Additional studies are in progress throughout Illinois to assess incidence rates and the possibility of geographical variation in vancomycin-susceptible isolates.

We thank John Earhart, Geraldine Thomas, Charlotte Arnold, Mark Ekwood, Mohammud Yousuf, and Rita Brenden for technical assistance, and John Meitl for encouragement. We thank Burroughs Wellcome Co, Research Triangle Park, North Carolina; Upjohn Co, Kalamazoo, Michigan; and Merck, Sharp and Dohme, Rahway, New Jersey, for their generosity.

References

1. Martin JE, Armstrong JH, Smith PB. New system for cultivation of *Neisseria gonorrhoeae. Appl Microbiol* 1974; 27: 802-5.
2. Thayer JD, Martin JE. A selective medium for the cultivation of *N gonorrhoeae* and *N meningitidis. Public Health Rep* 1964;79:49-57.
3. Martin JE jun, Lewis J. Anisomycin: improved antimicotic activity in modified Thayer-Martin medium. *Public Health Lab* 1977;35:53-60.
4. Smeltzer MP, Curran JW, Lossick JG. A comparative evaluation of media used to culture *N gonorrhoeae. Public Health Lab* 1979;37:43-56.
5. Taylor E, Phillips I. Assessment of a selective medium for the isolation of *Neisseria gonorrhoeae. Br J Vener Dis* 1979;55:183-5.
6. Reyn A. Recent developments in the laboratory diagnosis of gonococcal infections. *Bull WHO* 1969;40:245-55.
7. Reyn A, Bentzon MW. Comparison of a selective and non-selective medium in the diagnosis of gonorrhoea to ascertain the sensitivity of *Neisseria gonorrhoeae* to vancomycin. *Br J Vener Dis* 1972;48:363-8.
8. Cross RC, Hoger MB, Neibaur R, Pasterneck B, Brady F. VCN-inhibited strains of *Neisseria gonorrhoeae. HSMHA Hlth Rep* 1971;86:990-2.
9. Brorson JE, Holmberg I, Nygren B, Seaberg S. Vancomycin-sensitive strains of *Neisseria gonorrhoeae.* A problem for the diagnostic laboratory. *Br J Vener Dis* 1973;49:452-3.
10. Phillips I, Humphrey D, Middleton A, Nicol CS. Diagnosis of gonorrhoea by culture on a selective medium containing vancomycin, colistin, nystatin, and trimethoprim (VCNT). A comparison with Gram staining and immunofluorescence. *Br J Vener Dis* 1972;48:287-92.
11. Flynn J, Waitkins SA. A serum-free medium for testing fermentation reactions in *Neisseria gonorrhoeae. J Clin Pathol* 1972;25:525-7.
12. Miller MA, Scharli CS, Mills J. Cytoplasmic membrane proteins of spectinomycin-susceptible and -resistant strains of *Neisseria gonorrhoeae. J Bacteriol* 1980;143:198-204.
13. Guerry P, LeBlanc D, Falkow S. General method for the isolation of plasmid deoxyribonucleic acid. *J Bacteriol* 1973;116:1064-6.
14. Meyers J, Sanchez D, Elwell L, Falkow S. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. *J Bacteriol* 1976;127:1529-37.
15. Mickel S, Arena V jun, Bauer LS. Physical properties and gel electrophoresis behavior of RIZ-derived plasmid DNAs. *Nucleic Acids Res* 1977;4:1465-82.
16. Foster R, Foster G. Electrophoretic comparison of endonuclease-digested plasmids from *Neisseria gonorrhoeae. J Bacteriol* 1976;126:1297-304.
17. Phillips I, Ridley M, Rimmer D, Lynn R. In-vitro activity of 12 antibacterial agents against *Neisseria gonorrhoeae. Lancet* 1970;i:263-5.
18. Darrell JH, Garrod LP, Waterworth PM. Trimethoprim: laboratory and clinical studies. *J Clin Pathol* 1968;21:202-9.
19. Phillips I, Humphrey D, Middleton A, Nicol CS. Diagnosis of gonorrhoea by culture on a selective medium containing vancomycin, colistin, nystatin, and trimethoprim (VCNT): A comparison with Gram-staining and immunofluorescence. *Br J Vener Dis* 1972;48:287-92.
20. Windall JJ, Hace MM, Washington II JA, Douglass TJ, Weed LA. Inhibitory effects of vancomycin on *Neisseria gonorrhoeae* in Thayer-Martin medium. *J Infect Dis* 1980;142:775.
21. Knapp JS, Holmes KK. Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. *J Infect Dis* 1975;132:204-8.
22. Sackel SG, Alpert S, Rosner B, McLormack WM, Findland M. In-vitro activity of p-hydroxybenzyl penicillin (penicillin X) and five other penicillins against *Nesseria gonorrhoeae*: comparisons of strains from patients with uncomplicated infections and from women with pelvic inflammatory disease. *Antimicrob Agents Chemother* 1977;12:31-6.
23. Weisner PJ, Handsfield HH, Holmes KK. Low antibiotic resistance of gonococci causing disseminated infection. *N Engl J Med* 1973;288:1221-2.
24. Thompson S, Reynolds G, Short H, *et al.* Auxotypes and antibiotic susceptibility patterns of *Neisseria gonorrhoeae* from disseminated and local infections. *Sex Transm Dis* 1978;5:127-31.
25. Reynolds G, Ziadi A, Thornsberry C, Biddle J, Wiesner P. The National Gonorrhea Therapy Monitoring Study: II Trends and seasonality of antibiotic resistance of *Neisseria gonorrhoeae. Sex Transm Dis* 1979;6 suppl: 103-11.
26. Mayer LW, Holmes KK, Falkow S. Characterization of plasmid deoxyribonucleic acid from *Neisseria gonorrhoeae. Infect Immun* 1974;10:712-7.
27. Elwell LP, Falkow S. Plasmids of the genus *Neisseria.* In: *The Gonococcus,* Roberts RB, ed. New York: John Wiley & Sons, 1977;138-54.
28. Roberts M, Elwell L, Falkow S. Introduction to the mechanisms of genetic exchange in the gonococcus: Plasmids and conjugation in *Neisseria gonorrhoeae.* In: Brooks G, Gotschlich E, Holmes K, Sawyer W, Young F eds. *Immunobiology of Neisseria gonorrhoeae.* Washington DC: American Society for Microbiology, 1978;38-43.
29. Sparling PF, Guymon L, Biswas G. Antibiotic resistance in the gonococcus. In: Schlessinger D ed. *Microbiology – 1976.* Washington DC: American Society for Microbiology, 1976;44-52.
30. Maness M, Sparling PF. Multiple antibiotic resistances due to a single mutation in *Neisseria gonorrhoeae. J Infect Dis* 1973;128:321-30.

Br J Vener Dis 1982;**58**:101-4

Treatment of pharyngeal gonorrhoea due to β-lactamase-producing gonococci

MAGNUS LINDBERG,* OLOF RINGERTZ,† AND ERIC SANDSTRÖM*

*From the Departments of *Dermatology and †Clinical Bacteriology, Karolinska Institute, Södersjukhuset, Stockholm, Sweden*

SUMMARY Between March 1978 and October 1980 seven patients with β-lactamase-producing gonococci in the pharynx were treated with spectinomycin or cefuroxime or both. One-day treatment with spectinomycin was effective in only one of six patients and with cefuroxime in only one of four patients. Prolonged treatment with cefuroxime was successful in all five cases so treated.

Introduction

During the last few years an increasing number of genital infections caused by β-lactamase-producing gonococci (PPNG) has been found in Sweden.[1] Most of these have been contracted abroad, usually in the Far East. So far there has only been a limited spread within Sweden and most of the indigenous cases have been direct contacts of a patient infected abroad.

Gonococcal infections of the pharynx are not uncommon among patients with genital gonorrhoea and are seen among both heterosexuals and homosexuals.[2] During 1980 gonococci were isolated from the pharynx in 110 out of the 795 (14%) patients treated for gonorrhoea in our clinic.

When the first patient with pharyngeal gonorrhoea caused by PPNG attended our clinic in 1978, there was some uncertainty about treatment, and various regimens were tried. This study reports the symptoms and treatment of patients with such infections.

Patients and methods

STUDY POPULATION

At the venereal disease clinic at Södersjukhuset, Stockholm, β-lactamase-producing *Neisseria gonorrhoeae* were isolated from 17 patients between March 1978 and October 1980. Cultures were generally taken from the urethra, cervix, rectum, and pharynx of women and from the urethra and pharynx of men. Cultures from the pharynx were obtained from all patients infected with PPNG strains; PPNG were isolated from this site in five men and three women. One man did not complete his treatment and was excluded from the study.

All four men had contracted their infections in Thailand, one woman was infected in Portugal, and the remaining two were infected in Sweden by men who had recently been abroad in Thailand and Spain.

After treatment the patients were usually followed for a period of four weeks and cultures performed at least four times.

CULTURE TECHNIQUES

Specimens were taken with cotton swabs treated with charcoal. Each swab was immediately inoculated on to GC agar medium, consisting of Columbia blood agar base (Oxoid CM 331) supplemented with blood heated to approximately 80°C and with sterile GC supplement (Oxoid SR 56) containing vancomycin, colistin, trimethoprim, and nystatin, and on a medium of the same composition, to which sterile GC antibiotic supplement without antibiotics (Oxoid SR 62) had been added. The plates were immediately incubated at 37°C in a candle jar for a few hours before transport to the bacteriological laboratory in an adjoining building, where incubation was continued at 37°C in an atmosphere of 5% CO_2 for 48 hours. The plates were read by a plate microscope. Gonococci were identified by the oxidase test, microscopy, fermentation of dextrose but not maltose and levulose, and the coagglutination test (Phadebact, Pharmacia).

SENSITIVITY TESTING

Sensitivity tests were carried out by the agar diffusion method[3] on GC agar medium using antibiotic discs (Biodisk, Stockholm). All isolates were tested for β-lactamase production with chromogenic cephalosporin (Biodisk, Stockholm, Sweden) and, for most strains, also by modified Escamilla[4] and clover leaf[5] techniques.

Address for reprints: Dr O Ringertz, Department of Clinical Bacteriology, Södersjukhuset, S-100 64 Stockholm 38, Sweden

Accepted for publication 26 October 1981

Results

SENSITIVITY TO ANTIBIOTICS

The sensitivity pattern of the *N gonorrhoeae* strains isolated from the seven patients with pharyngeal infection is summarised in table I. The sensitivity pattern was similar for most of the strains. All were found to produce β-lactamase by all three methods used. They were all sensitive to spectinomycin and cefuroxime.

TABLE I *Antibiotic sensitivity (MIC) of seven β-lactamase-producing strains of N gonorrhoeae isolated from pharyngeal swabs*

Antibiotic	*MIC range (μg/ml)*
Benzylpenicillin	>8
Ampicillin	>16
Cephalosporin	4-8
Erythromycin	0·25-1
Clindamycin	0·5-4
Tetracycline	0·125-2
Chloramphenicol	1-16
Gentamicin	2-64
Fusidic acid	4-16
Spectinomycin	8-16
Cefuroxime	0·25-1

In one patient two different β-lactamase-producing strains were isolated. They differed significantly in their sensitivity to erythromycin, lincomycin, and rifampicin.

CLINICAL SYMPTOMS

The clinical symptoms reported by the seven patients are summarised in table II.

TABLE II *Clinical symptoms in seven patients with β-lactamase-producing N gonorrhoeae in pharyngeal cultures*

Symptoms	*Men (4)*	*Women (3)*	*Total*
Genital discharge	4	2	6
Fever	2	2	4
Sore throat	2	1	3
Arthralgia	0	1	1
Epididymitis	1	0	1
None	0	1	1

TREATMENT

The standard treatment for gonorrhoea given in our clinic consists of pivampicillin (1·4 g) with probenecid (1·0 g) at the first visit if the microscopy results are positive. At the second visit one week later the treatment is adjusted according to the results of the culture and sensitivity test carried out at the first visit. Six of the patients with PPNG in the pharynx were thus initially treated with pivampicillin.

Regimens (A) and (B)

During the first part of the study spectinomycin was usually given at the second visit, but later when cefuroxime became available this drug was introduced for the treatment of infections with PPNG. Owing to the therapeutic failures different doses have been given and the same patients have sometimes received several courses of treatment. The treatment schedules have been classified as 'one day' treatment (A) or 'more than one day' treatment (B). The results are summarised in table III.

TABLE III *Results of different treatment regimens*

	No of cures/total No of treatments for gonorrhoea of:	
Treatment	*Pharynx*	*Urethra*
Regimen (A)*		
Pivampicillin		
1·4g or 2·45g plus probenecid 1·0g	0/5	0/4
Spectinomycin		
2·0g	0/3	3/3
2·0g × 3 doses	1/2	1/1
4·0g	0/1	
Total	1/6	4/4
Cefuroxime		
1·5g	0/1	
0·75g + probenecid 1·0g	0/1	
1·0g + probenecid 1·0g	1/1	
1·5g × 2 doses	0/1	
Total	1/4	
Regimen (B)*		
Pivampicillin		
0·35g × 3 doses daily for 7 days	0/1	0/1
Spectinomycin		
2·0g daily for 3 days	0/1	1/1
Cefuroxime		
1·5g daily for 3 days	1/1	
1·5g + probenecid 1·0g daily for 3 days	1/1	1/1
0·75g daily for 3 days	1/1	
0·75g + probenecid 1·0g daily for 3 days	1/1	
1·5g × 3 doses daily for 7 days	1/1	
Total	5/5	1/1

Regimen (A) = treatment for one day; regimen (B) = treatment for more than one day

Treatment (A) with spectinomycin cured the pharyngeal infection in only one of six cases; for cefuroxime the corresponding figure was one out of four. Treatment (A) with spectinomycin, however, cured the urethral infection in all of four patients.

Treatment (B) with spectinomycin was given to only one patient with pharyngeal infection, but without success. Treatment (B) with cefuroxime was successful in all of five cases.

TEST-OF-CURE CULTURES

The patients were considered to be cured when at least four culture results were negative during a period of at least four weeks. In five inpatients throat swabs for culture were usually taken three times a day during treatment and for the following few days. Treatment with spectinomycin or cefuroxime gave negative culture results within a few hours of injection. In most cases of therapeutic failure the culture results became positive again within less than a week. In one case, however, gonococci were not isolated until the fourth culture taken three weeks after treatment (figure). In none of the cases were the positive culture results after treatment believed to be due to reinfection.

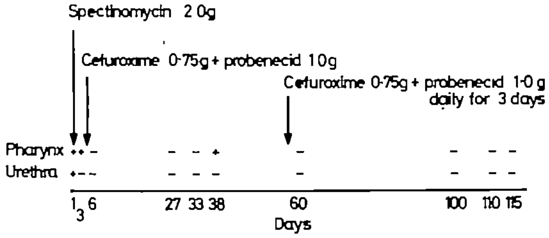


FIGURE *Results of cultures and treatment given to one patient.*

Discussion

Pharyngeal infections with penicillin-sensitive *N gonorrhoeae* are more difficult to treat than anogenital gonorrhoea. Bro-Jörgensen and Jensen[6] reported that only 60% of their 93 patients were cured by a single dose of ampicillin, pivampicillin, or penicillin G with probenecid compared with almost 100% of patients with anogenital infections. This agrees with our own experience.

Wiesner *et al*[7] reported that spectinomycin had a poor effect on pharyngeal gonorrhoea caused by non-PPNG strains. These results were not confirmed in the five patients with pharyngeal infection caused by PPNG strains in the US PPNG surveillance study.[8] In the present study different spectinomycin regimens were, therefore, tried before cefuroxime became available. Our results indicate that although treatment with a single dose of spectinomycin may be sufficient to cure an urethral infection with PPNG, the pharyngeal infection is usually not cured. Our data do not allow any conclusions about the effectiveness of prolonged treatment with spectinomycin.

Jones *et al*[9] reported that one-day treatment with 1 g or 2 g of cefoxitin was not sufficient to eradicate oropharyngeal carriage of *N gonorrhoeae*, although the urogenital infection was cured in all 25 cases studied.

Moberg and Wennberg[10] reported successful treatment of 38 out of 39 female patients with urogenital gonorrhoea with 1 g of cefuroxime intramuscularly in combination with 1 g probenecid orally.

In the present study only one of four patients with pharyngeal gonorrhoea was cured by a single dose of cefuroxime. When the treatment with cefuroxime was extended for three days or more, however, all five patients were cured of pharyngeal gonorrhoea clinically and bacteriologically.

The importance of repeated cultures after treatment is clearly seen in our study. After treatment with both spectinomycin and cefuroxime culture results may initially be negative only to become positive later.

Although there is a tendency to regard gonococci in the pharynx as colonisation without clinical importance,[11] Wiesner *et al*[7] found an increased incidence of pharyngeal infection among heterosexual men with disseminated gonococcal infection. In this context it is of interest that four of seven of our patients with pharyngeal infection caused by PPNG strains had fever, one had arthralgia, and one epididymitis, although PPNG strains are not considered to be more virulent than non-PPNG strains.[8]

The high incidence of pharyngeal infection in patients with PPNG (47% in our series) is of concern because of the coexistence of meningococci in patients with pharyngeal gonococcal infection reported by Noble *et al.*[12] Experimentally, genetic information is readily exchanged between different *Neisseria* species[13] and the transfer of the β-lactamase plasmid to meningococci is a possibility. Even if the epidemiological significance of the presence of gonococci in the pharynx is not clear[11] it, therefore, seems reasonable to eliminate the PPNG strains as quickly as possible.

From our experience we propose the following management of patients with anogenital PPNG strains: (a) at least two cultures should be obtained from the pharynx before a pharyngeal infection is excluded; (b) patients with a pharyngeal PPNG infection should be treated with 1500 mg cefuroxime intramuscularly once daily for three days or 750 mg cefuroxime intramuscularly and 1 g probenecid orally once daily for three days; and (c) after treatment of pharyngeal infection four negative culture results should be obtained over a four-week period to confirm complete cure.

References

1. Danielsson, D, Moi H, Olovsson L. Penicillinresistent gonorré —Skärpt kontroll krävs. *Läkartidningen* 1980;77:4623-6.
2. Osborn NG, Grubin, L. Colonization of the pharynx with *Neisseria gonorrhoeae. Sex Transm Dis* 1979;6:253-6.

3. Eriksson H, Sherris JC. Antibiotic sensitivity testing. Report of an international collaborative study. *Acta Pathol Microbiol Scand (B)* 1971; suppl 217: 1-90.
4. Escamilla J. Susceptibility of *Haemophilus influenzae* to ampicillin as determined by the use of a modified, one-minute beta-lactamase test. *Antimicrob Agents Chemother* 1976;**9**: 196-8.
5. Örstavik I, Ödegaard K. A simple test for penicillinase production in *Staphylococcus aureus*. *Acta Pathol Microbiol Scand (B)* 1971;**79**:855-6.
6. Bro-Jörgensen A, Jensen T. Gonococcal pharyngeal infections. Report of 110 cases. *Br J Vener Dis* 1973;**49**:491-9.
7. Wiesner PJ, Tronca E, Bonin P, Pedersen AHB, Holmes KK. Clinical spectrum of pharyngeal gonococcal infection. *New Engl J Med* 1973;**288**: 181-5.
8. Siegel MS, Thornsberry C, Biddle JW, *et al.* Penicillinase-producing *Neisseria gonorrhoeae:* Results of surveillance in the United States. *J Infect Dis* 1978;**137**:170-5.
9. Jones RB, Stimson J, Counts GW, Holmes KK. Cefoxitin in the treatment of gonorrhoea. *Sex Transm Dis* 1979;**6**:239-42.
10. Moberg I, Wennberg K. Treatment of females with acute gonorrhoea using cefuroxime. *Proc R Soc Med* 1977;**70** suppl 9: 128-30.
11. Wallin J, Siegel MS. Pharyneal *Neisseria gonorrhoeae:* coloniser or pathogen? *Br Med J* 1979; i: 1462-3.
12. Noble TC, Cooper RM, Miller BR. Pharyngeal colonisation by *Neisseria gonorrhoeae* and *Neisseria meningitidis* in black and white patients attending a venereal disease clinic. *Br J Vener Dis* 1979;**55**: 14-9.
13. Catlin BW. Genetic studies of sulfadiazine-resistant and methionine-requiring *Neisseria* isolated from clinical material. *J Bacteriol* 1967;**94**:719-33.

Br J Vener Dis 1982;**58:**105-8

Effects of tampon components on growth and dissemination of Neisseria gonorrhoeae

R J ARKO, K H WONG, K G FINLEY-PRICE, AND J K RASHEED
From the Center for Infectious Diseases, Center for Disease Control, Atlanta, Georgia, USA

SUMMARY Six components used in vaginal tampons were tested for their effects on a strain of *Neisseria gonorrhoeae* isolated from a patient with disseminated infection. Tampon components containing carboxymethyl cellulose or its derivative prolonged the in-vitro survival of gonococci and, when injected with mucin into mice, significantly ($P<0\cdot0001$) increased the dissemination of gonococci from the peritoneal cavity. In contrast, a component extracted from rayon tampons reduced in-vitro survival and appeared to suppress gonococcal dissemination in mice. Since tampons are used by a large number of women at a time when the risk of developing complications from venereal infections are increased, their effects on potential urogenital pathogens warrant further study.

Introduction

The association of toxic shock syndrome (TSS) in menstruating women with vaginal *Staphylococcus aureus* and the use of tampons[1-3] has stimulated interest in determining the biological effects of various tampon components on other pathogens associated with the female reproductive tract. The use of intrauterine contraceptive devices (IUCDs) has already been identified as a risk factor for women in developing pelvic inflammatory disease (PID) due to ascending infections with *Neisseria gonorrhoeae, Chlamydia trachomatis,* and possibly other pathogens.[4-6] Menstruation also appears to increase the risk of complications of PID and disseminated gonococcal infection (DGI). Whether this is due to physiological changes accompanying menses or to a combination of intrinsic and extrinsic factors, such as IUCDs and tampons, remains to be determined.[4 7] This study was undertaken to determine the effects of selected tampon components on in-vitro growth and survival and on in-vivo dissemination of *N gonorrhoeae* in laboratory animals.

Materials and methods

BACTERIA

A strain of *N gonorrhoeae* isolated from the joint fluid of a patient with DGI was used for testing tampon materials. Stock cultures of this strain were frozen at −60°C until streaked on to a GC base medium supplemented with 1% IsoVitalex (BBL-Microbiology Systems, Cockeysville, MD). After incubation at 36°C for 20 hours in a candle-extinction jar, the gonococci were harvested with a cotton swab into sterile phosphate-buffered (0·067 mol/l KH_2PO_4 and Na_2HPO_4) saline (PBS), pH 7·4. A spectrophotometer set at 540 nm was used to adjust cell suspensions to an optical transmittance of 50%. Serial 10-fold dilutions of the cell suspension were then quantitated in colony-forming units (cfu) by plate counts. The cell suspensions for inoculating animals contained gonococci from 10^5 to 10^8 cfu/ml.

TAMPONS

Two commercial tampons* and six tampon component materials were tested. The Rely tampon (lot 15501A16A, Proctor and Gamble, Cincinnati, Ohio) and the Tampax Super Plus tampon (lot 6T072T, Tampax Inc, Palmer, Mass) were purchased from local stores. Components of the tampons tested included: from Rely tampons (a) cross-linked derivative of carboxymethyl cellulose (CLD-CMC) and (b) polyester foam pieces; from Tampax tampons (a) powdered carboxymethyl cellulose (CMC) 'Aqualon', (b) rayon 8285, (c) 'Fiber-T-/MV/CL/Comber/Aqualon', and (d) 'Fiber-T-/MV/CL/ Comber.'

Address for reprints: Dr R J Arko, Building 1, Room 3254, Center for Disease Control, Atlanta, Georgia 30333, USA

Accepted for publication 29 October 1981

---

*Use of trade names and commercial sources is for identification only and does not constitute endorsement by the Public Health Service or by the US Department of Health and Human Services.

IN-VITRO TESTS

Tampon components

Aqueous suspensions containing 1-2% (wt/vol) of tampon material were prepared in PBS pH 7·4 and tested for inhibitory activity to *N gonorrhoeae*. Suspensions were tested both before and after filtration through a Millipore membrane with a porosity of 0·22μ (Millipore Co, Bedford, Mass). Non-filtered suspensions were also tested after being boiled for 30 minutes. Drops of 0·05 ml of tampon suspensions were absorbed into areas of about 1·0 cm in diameter on GC base plates or suspensions were pipetted into wells (5·0 mm in diameter) cut into the agar surface. A lawn of 10^4 cfu of gonococci suspended in 0·05 ml of PBS was then streaked over the prepared plates. After incubation for 20 hours the plates were examined under a stereoscopic microscope to determine the growth pattern in the presence and absence of the tampon suspension.

Whole tampons

The leachability and pH of Rely (CLD-CMC) and Tampax Super Plus (Rayon 8285) tampons were determined after suspending each tampon in 50·0 ml of PBS, pH 7·4. The turbidity imparted by material leached from each tampon was measured at ½-hour, 1½-hour, and three-hour intervals with a spectrophotometer set at 540 nm. Tampons were also compared for their effects on the attachment of mouse peritoneal macrophages and on the survival of gonococcal cells. For these tests, tampons were suspended in glass containers with 100 ml of Hanks balanced salts (HBS) solution (pH 7·0). Each tampon was tested in triplicate along with HBS controls (without tampon). Mouse macrophages, collected by intraperitoneal lavage of mineral oil-primed mice, were washed in HBS and then added (10^5 cells) to each tampon and control suspension. The number of macrophages remaining 'free' in each suspension was determined by cell counts made at ½-hour, three-hour, six-hour, and 24-hour intervals after inoculation. In addition to the mouse macrophages, 10^8 cfu of *N gonorrhoeae* were added to each suspension. Survival of *N gonorrhoeae* was determined by sequential cultures made from 0·05-ml aliquots of each suspension at three-hour, six-hour, and then 24-hour intervals until no growth was obtained.

IN-VIVO TESTS

The ability of tampon materials to enhance or suppress the circulatory dissemination of gonococci was tested in a mouse intraperitoneal infection model.[8-10] Granular mucin and bovine haemoglobin were prepared as described[8] and included in the challenge inoculum where indicated. The challenge inocula were prepared as follows: (a) 9·3 mg of mucin (type 1701-W, Wilson Laboratories, Chicago, Ill) and 3·3 mg of haemoglobin (Difco Laboratories, Detroit, Michigan) were mixed with gonococci and tampon materials in a final volume of 0·5 ml/challenge dose; (b) 16·6 mg of mucin was mixed with gonococci and tampon materials. Immediately after mixing, the inocula were injected intraperitoneally into 3-6-week-old Institute of Cancer Research (ICR) white mice. Twenty-four hours later the challenged mice were narcotised by inhalation of CO_2, and 0·2 ml of heart blood was aspirated from each. Approximately 0·1 ml of each specimen was immediately streaked on to plates of GC base medium. After incubation in a candle jar for 24 hours, each plate was examined and the approximate number of cfu of gonococcal growth noted (tables I and II).

ANALYSIS OF DATA

The infectious dose 50% (ID_{50}) was determined by interpolation between doses of gonococci giving less than and greater than 50% positive blood culture results. Statistical comparisons were made by the χ^2 test.[11]

TABLE I *Effects of tampon components on dissemination of Neisseria gonorrhoeae in mice challenged intraperitoneally*

Tampon component mixed with challenge*	*Challenge dose of gonococci (cfu)* 10^6†	10^7‡	10^8	*Infectious dose 50%*
(A) Aqualon CMC powder	7/30	25/43	18/19	$10^{6·77}$
(B) CLD-CMC	0/28	27/46	NT	$10^{6·85}$
(C) Polyester foam	NT	12/27	NT	$>10^{7·0}$
(D) Rayon No 8285	0/20	9/36	32/46	$10^{7·56}$
(E) Fiber-T-/MV/ CL/Comber/Aqualon	0/10	10/16	45/48	$10^{6·80}$
(F) Fiber-T-/MV/CL/Comber	0/18	6/16	43/48	$10^{7·24}$
(G) Phosphate buffered saline-mucin control	0/38	19/51	12/18	$10^{7·43}$

*2·0 mg of tampon material plus 16·6 mg of mucin in 0·5 ml PBS.
†0·1 ml of heart blood cultured from each mouse 24 h after inoculation (number with positive blood culture/number cultured).
‡χ^2 values: A+B+E *vs* D+F+G = $P<0·0001$, A+B *vs* D = $P<0·01$, and D *vs* G = $0·2<P<0·3$.
cfu = colony-forming units; NT = no test.

TABLE II *Effects of tampon components on mortality and gonococcal bacteraemia in mice given intraperitoneal inoculations*

*Inoculum components**	*% Mortality†*	*No cultured*	*% Mice having indicated cfu of gonococci per ml of blood‡*			
			0	*1-400*	*401-800*	*>800*
PBS diluent control	0	26	100	0	0	0
CLD-CMC	0	32	69	25	3	3
Bovine haemoglobin plus mucin	23	54	50	39	2	9
CLD-CMC plus bovine haemoglobin and mucin	25	76	25	34	8	33

*Inoculum (0·5 ml) contained: 10^7 cfu of *N gonorrhoeae*, 2·0 mg of cross-linked derivative of carboxymethyl cellulose (CLD-CMC), 3·3 mg of bovine haemoglobin, 9·3 mg gastric mucin suspended in phosphate-buffered saline (PBS), pH 7·4, PBS substituted for components not in each test.

†Mice dying in <24 h and not cultured.

‡Approximately 0·1 ml of heart blood cultured for *N gonorrhoeae* 24 h after inoculation.

Results

Aqueous extracts of tampons or their components showed significant variation in their effects on the growth, survival, and in-vivo dissemination of *N gonorrhoeae*. Inocula containing mucin plus tampon components of CLD-CMC or Aqualon CMC produced significantly (P<0·0001) higher rates of gonococcal bacteraemia in mice than in mucin controls and in groups not receiving CMC (table I). The CLD-CMC component when combined with mucin and haemoglobin produced a significantly (P<0·001) higher systemic gonococcal infection in mice than did control inocula without CLD-CMC (table II). Rayon extracts appeared to suppress the in-vivo dissemination of *N gonorrhoeae* in the mouse model. Significantly fewer (P<0·01) mice in group D (rayon 8285) developed bacteraemia than did those in groups A and B receiving CMC or its derivative (table I). Although fewer mice in group D (9/36) became bacteraemic than in group G (PBS-mucin controls, 19/51), this difference was not significant (P0·2<P<0·3).

When rayon and CLD-CMC tampons were compared for leachability and pH, and for their effects on gonococci and the attachment of mouse macrophages, (a) CLD-CMC tampons produced substantially higher levels of turbidity in suspensions with PBS; percentage optical transmittance ranged from 20-23% with CLD-CMC tampons compared with 79-91% for rayon tampons and 100% for PBS controls; (b) suspensions of rayon tampons were slightly more acid, pH 6·7 compared with pH 7·0 for CLD-CMC tampons; (c) by indirect determinations macrophages appeared to attach more rapidly to the rayon tampons (figure, *a*); and (d) gonococci remained viable for a substantially shorter period in suspension with rayon tampons than in the control HBS or CLD-CMC suspensions (figure, *b*). In addition, gonococci growing on a translucent medium showed phenotypic changes in morphology from smooth transparent to granular opaque colonies in areas where the rayon extract had been absorbed. A similar predominance of opaque colonies occurred around agar wells containing the rayon extract.

The factor(s) in rayon extracts producing the effects on gonococcal cells appeared to be heat stable, resisting boiling for 30 minutes, and were filterable through a 0·22-μ Millipore membrane. Analysis of these extracts by gas-liquid chromatography failed to detect fatty acids (C W Moss, unpublished data).

Discussion

The purpose of this study was to determine the biological effects and possible mechanisms whereby tampon materials may influence the growth and dissemination of *N gonorrhoeae*. Several mechanisms potentially affecting the virulence of gonococci became apparent. (a) The CMC components appeared more soluble in aqueous fluids and had a greater tendency to leach out of the tampon into the surrounding fluid, possibly producing conditions favourable to the survival of gonococci. (b) The wrapped CLD-CMC tampons appeared to provide a less effective surface for the attachment of macrophages than did the non-wrapped rayon tampon. This could conceivably leave a greater number of macrophages engorged with gonococci after removal of the tampon from the vagina. The intracellular survival of gonococci in macrophages may thus act as a 'Trojan horse' for the dissemination of the infection across otherwise impregnable tissues.[12] (c) Certain tampon components may inhibit or alter the growth of gonococci, rendering cells more susceptible to host defences. Cells from rough or opaque

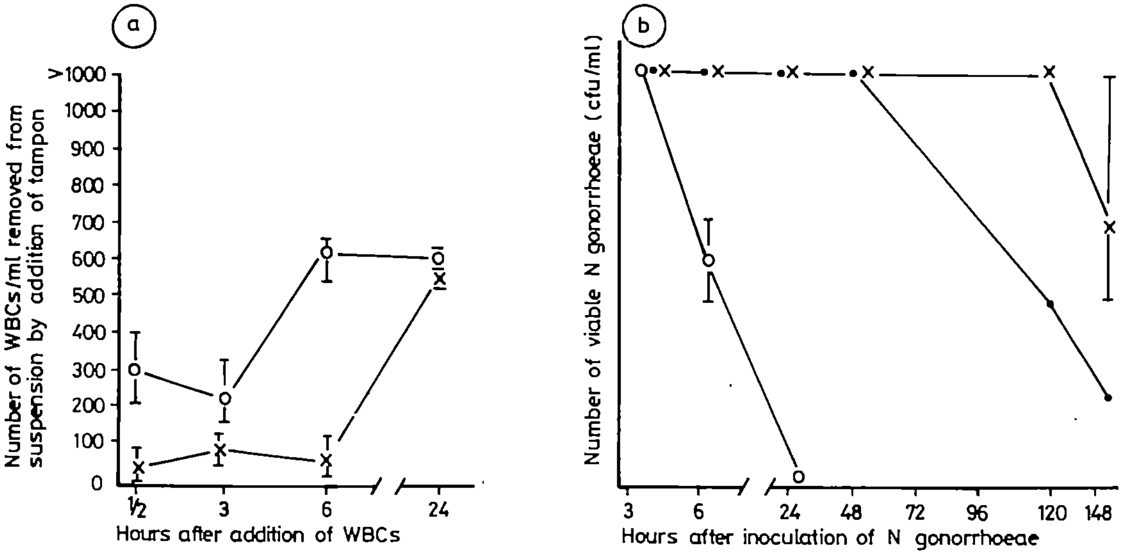


FIGURE *Comparison of non-wrapped rayon tampon (○) with one of wrapped cross-linked derivative of carboxymethyl cellulose (×) for effects on (a) attachment of mouse macrophages (WBC) and (b) survival of Neisseria gonorrhoeae in tampon fluid (● – ● diluent control, 100 ml of Hanks balanced salt solution).*

colonies, similar to those induced by rayon extracts, can be approximately four times less resistant to normal human serum than cells of smooth or transparent colonies.[13]

The practical implications of these differences in promoting or inhibiting the dissemination of *N gonorrhoeae* by tampon materials remains to be ascertained. The effects of tampons on pathogenic micro-organisms in the vagina, however, warrants further study because a number of serious disease complications may arise from this anatomical site.

The authors thank D C Mackel, R L Anderson, and members of the CDC toxic shock task force for helpful discussion; W O Schalla for technical help; and S J Smith for statistical advice.

References

1. Davis JP, Chesney PJ, Wand PJ, *et al.* Toxic shock syndrome: epidemiologic features, recurrence, risk factors, and prevention. *N Engl J Med* 1980;**303**:1429-35.
2. Shands KN, Schmid GP, Dan BB, *et. al.* Toxic-shock syndrome in menstruating women: its association with tampon use and *Staphylococcus aureus* and the clinical features in 52 cases. *N Engl J Med* 1980;**303**:1436-42.
3. Todd J, Fishaut M, Kapral F, Welch T. Toxic shock syndrome associated with phage-group-1 staphylococci. *Lancet* 1978;ii:1116-8.
4. Burkman RT. Intrauterine device use and risk of pelvic inflammatory disease. *Am J Obstet Gynecol* 1980;**138**:861-3.
5. Holmes KK, Counts GW, Beaty HN. Disseminated gonococcal infection. *Ann Intern Med* 1971;**74**:979-93.
6. Scott RB. Critical illness and death associated with intrauterine devices. *Obstet Gynecol* 1968;**31**:322-5.
7. Rees E, Annels EH. Gonococcal salpingitis. *Br J Vener Dis* 1977;**45**:205-15.
8. Corbeil LB, Wunderlich AC, Corbeil RR, McCutchan JA, Ito JI Jun, Brande AI. Disseminated gonococcal infection in mice. *Infect Immun* 1979;**26**:984-90.
9. Keefer CS, Spinks WW. Studies on gonococcal infection. IV The effect of mucin on the bacteriolytic power of whole blood and immune serum. *J Clin Invest* 1938; **17**:23-30.
10. Miller CP, Hawk WD. Experimental infection of mice with gonococcus. *Arch Pathol* 1939;**28**:764-9.
11. Snedecor GW, Cochran GW. *Statistical Methods*, 6th ed. Ames, Iowa: The Iowa University Press, 1967;218.
12. Novotny P, Short JA, Hughes M, *et al.* Studies on the mechanism of pathogenicity of *Neisseria gonorrhoeae*. *J Med Microbiol* 1977;**10**:347-65.
13. James JF, Swanson J. Color/opacity colonial variants of *Neisseria gonorrhoeae* and their relationship to the menstrual cycle. In: Brooks GF, Gotschlich EC, Holmes KK, Sawyer WD, Young FE, eds. *Proceedings of Immunobiology of Neisseria gonorrhoeae*. Washington DC: American Society for Microbiology, 1978:338-43.

Br J Vener Dis 1982;**58**:109-12

Rapidity and reliability of gonococcal identification by coagglutination after culture on modified New York City medium

H YOUNG* AND A McMILLAN†

*From the *Department of Bacteriology, Edinburgh University Medical School and Royal Infirmary; and the †University Department of Genitourinary Medicine, Royal Infirmary, Edinburgh*

SUMMARY The combination of culture on modified New York City (MNYC) medium and identification of neisserial isolates by the Phadebact® gonococcus test was evaluated in routine laboratory practice. The sensitivity of coagglutination was 96·7% (318/329 isolates) and that of immunofluorescence (IF) 97·9% (322/329 isolates); the specificity of both methods was 96·8% (120/124 isolates). Of the 329 gonococcal isolates, 286 (86·9%) could be tested by coagglutination and 309 (93·9%) by IF after only 24 hours' incubation. Identification by coagglutination from primary cultures on MNYC medium is considered to be very rapid, simple, and efficient for the cultural diagnosis of anogenital gonorrhoea in women and urethral gonorrhoea in men. Because of the high prevalence of meningococci in the pharynx and their not infrequent occurrence in the anorectum of homosexual men the identity of isolates from these sites is best confirmed by sugar utilisation tests.

Introduction

The Phadebact® gonococcus test (Pharmacia Diagnostics AB, Uppsala, Sweden) is a rapid slide test for the immunological identification of *Neisseria gonorrhoeae*. The test depends on the principle of coagglutination.[1] An early report from the United Kingdom[2] of gonococcal identification by coagglutination was critical of the specificity of the method. More recent reports from the United States,[3-5] Australia,[6] and Switzerland[7] suggest that coagglutination can provide rapid and accurate immunological confirmation of *N gonorrhoeae*.

Rapid cultural diagnosis of gonorrhoea, particularly in women, is of prime importance in the control of infection within the community. Modified New York City (MNYC) medium promotes rapid gonococcal growth and enables biochemical characterisation to be carried out directly from the primary isolation plate, providing a rapid and economical system of identification.[8] An improvement in the cultural diagnosis of gonorrhoea associated with the use of MNYC medium has been reported in the United Kingdom,[9] Norway,[10] and New Zealand.[11] The aim of the present study was to evaluate the combination of culture on MNYC medium with identification by the Phadebact® gonococcus test in routine laboratory practice.

Patients and methods

STUDY POPULATION

The patients in this study attended the department of genitourinary medicine at the Edinburgh Royal Infimary on a Monday, Tuesday, or Wednesday morning between November 1980 and May 1981. Investigations to detect or exclude gonococcal infection were as described.[9] All isolates of oxidase-positive Gram-negative diplococci were tested for penicillinase production and identified by the rapid carbohydrate utilisation test and by delayed immunofluorescence with Difco fluorescein-labelled anti-gonococcal conjugate.[8] The coagglutination test was performed as described below. Cultures giving negative results were re-examined after incubation for 48 hours.

Address for reprints: Dr H Young, Department of Bacteriology, Edinburgh University Medical School and Royal Infirmary, Teviot Place, Edinburgh EH8 9AG

Accepted for publication 26 October 1981

COAGGLUTINATION TEST

Lyophilised reagents

Commercially available test kits (Phadebact® gonococcus test) were provided by Pharmacia Diagnostics AB, Sweden. A heavy suspension of each culture to be tested was made by removing the growth from the culture plate with a cotton-tipped swab and emulsifying it in 0·2 ml distilled water in a tube. The tube was covered and the suspension heated in a boiling water-bath for five minutes. After cooling to room temperature 40-μl aliquots of suspension were mixed with an equal volume of test reagent (rabbit antigonococcal antiserum bound to dead staphylococci) and control reagent (non-immune rabbit serum bound to dead staphylococci) on a clear glass slide: three tests were carried out on a single slide (10·7 cm × 5·0 cm) with six clearly defined areas. The slide was rocked gently for two to three minutes by tilting to an angle of 45° every two to three seconds and the results read against a dark background using indirect light.

A markedly stronger reaction with the gonococcal reagent compared with the control reagent constituted a positive result. No reaction with the gonococcal reagent irrespective of any reaction in the control reagent was considered a negative result. A reaction of equal strength in both the gonococcal and the control reagents was classed as non-interpretable.

Isolates giving false-positive and false-negative results by sugar utilisation reactions were retested the next day as a 24-hour subculture. A suspension of growth from each subculture was also made in skimmed milk (10%) and stored frozen at −40°C.

Liquid reagents coloured with methylene blue

On completion of the trial isolates were reconstituted from the skimmed milk and tested with the new liquid reagents and with the conventional lyophilised reagents.

ANALYSIS OF RESULTS

Coagglutination and IF test results were correlated with sugar utilisation reactions. As the aim was to make an 'in use' evaluation, only gonococcal isolates giving clear-cut positive results with IF or coagglutination or both were scored positive; borderline reactions were scored negative. Conversely borderline reactions with non-gonococcal neisseriae were scored positive.

The sensitivity of coagglutination and IF methods was defined as the percentage of gonococcal isolates confirmed by carbohydrate utilisation tests that were IF-positive or coagglutination-positive, while specificity was defined as the percentage of non-gonococcal isolates confirmed by carbohydrate utilisation testing that was coagglutination-negative or IF-negative.

The significance of differences in the results with coagglutination and IF methods was determined by dividing the difference between the two totals to be compared by the standard error of the difference: a value equal to or greater than 1·96 indicates a significant difference at the 5% level.

Results

SPECIFICITY AND SENSITIVITY

There were 140 men (mean age 29·7 years) and 118 women (mean age 22·8 years) from whom oxidase-positive Gram-negative diplococci (GNDC) were isolated: 189 isolates of GNDC were obtained from 156 routine examinations of men and 264 isolates of GNDC from 131 routine examinations of women. The coagglutination, immunofluorescence (IF), and sugar utilisation results for the 453 isolates are shown in table I.

The sensitivity of the coagglutination test was 96·7% (318/329 isolates) and that of the IF test 97·9% (322/329 isolates). This difference is not

TABLE I *Results of coagglutination, immunofluorescence, and sugar utilisation tests applied to 453 neisserial isolates.*

Site	*No of cultures yielding oxidase-positive GNDC*	*Identification by sugar utilisation*			*Immunofluorescence*		*Coagglutination*	
		N gonorrhoeae	*N meningitidis*	*N lactamica*	*Positive*	*Negative*	*Positive*	*Negative*
Urethra	170	170			166	4	165	5
Cervix	89	88			86	2	85	3
			1		0	1	0	1
Rectum	49	48			47	1	45	3
			1		0	1	0	1
Bartholin's gland	8	8			8	0	8	0
Throat	137	15			15	0	15	0
			115		3	112	4	111
				7	1	6	0	7*
Total	453	329	117	7	326	127	322	131

GNDC = Gram-negative diplococci
*Includes one non-interpretable result

significant at the 5% level. The specificity of both coagglutination and IF was 96·8% (120/124 isolates). Four isolates of β-lactamase-producing gonococci gave positive results by both methods.

RAPIDITY OF DIAGNOSIS

Table II shows the rapidity of gonococcal identification by coagglutination and IF methods. The difference in the total number of isolates from all anatomical sites tested at 24 hours by each method was not significant at the 5% level.

Of the 115 meningococci isolated from throat cultures in men and women 96 (83·5%) were tested at 24 hours by coagglutination and 113 (98·3%) by IF.

FALSE-POSITIVE AND FALSE-NEGATIVE COAGGLUTINATION RESULTS

Seven isolates of gonococci (four from men and three from women) gave false-negative results when first tested. Only six of these isolates were available for retesting at the end of the trial and all gave a positive result. With the conventional freeze-dried reagents at least two minutes were required to produce coagglutination whereas this was clearly observable within 30 seconds with the liquid reagents containing methylene blue.

All four isolates of *N meningiditis* (two serogroup B and two non-groupable) that gave positive reactions initially gave negative results on retesting at the end of the survey.

Discussion

Our results indicate clearly that the overall sensitivity and specificity of coagglutination and IF are comparable. Similar results were obtained by Rufli[7] and by Johnston,[12] who prepared her own coagglutination reagents. We found the coagglutination method was simple to perform, easy to interpret, and does not require expensive immunofluorescence equipment. These advantages are of less benefit in laboratories with facilities for fluorescence microscopy and adequately staffed with skilled technologists. The cost of performing a coagglutination test with commercial reagents is approximately £0·50 when a 40-μl volume is used compared with a cost of £0·10 for an IF test with a 10-μl unit volume. If coagglutination can be performed equally well with a 10-μl volume, however, as reported by Futrovsky *et al*,[13] this would reduce the cost per test to approximately £0·125.

Both coagglutination and IF methods have the same disadvantages: the failure to detect a small proportion of gonococcal isolates (2-3%) and the failure to obtain a clear-cut negative reaction with a small proportion of non-gonococcal neisseriae. These problems can be minimised by using a carefully absorbed antigonococcal serum known to be reactive in the coagglutination test with local gonococcal strains.[12] Despite these limitations immunological identification is a very useful adjunct in the diagnostic laboratory. Confirmation of the identity of gonococci is obtained rapidly and with very small amounts of bacterial growth.

Provided that unequivocal results are obtained the specificity and sensitivity of immunological methods are such that further identification of urethral isolates from men and anogenital isolates from women is unnecessary provided that colonial morphology is typical and correlates with immunological findings. Whenever medico-legal proceedings may be involved full identification including sugar utilisation tests must be performed. Because meningococci are isolated from the throat much more often than gonococci, the small number of false-positive reactions that may occur decreases the utility of immunological identification of pharyngeal isolates.

Although there are several reports of the isolation of *N meningitidis* from anogenital sites,[14-16] when the

TABLE II *Proportion of gonococcal isolates from various anatomical sites tested by immunofluorescence and coagglutination after incubation of primary cultures for 24 hours*

Site	*No of isolates*	*No (%) of isolates tested at 24 hours by:*	
		Immunofluorescence	*Coagglutination*
Male urethra	106	104(98·1)	104(98·1)
Female urethra	64	56(87·5)	48(75·0)
Cervix	88	85(96·6)	79(89·8)
Male rectum	4	4*	4*
Female rectum	44	40(90·9)	37(84·1)
Bartholin's gland	8	8*	6*
Male throat	7	5*	3*
Female throat	8	7*	5*
Total	329	309(93·9)	286(86·9)

*Percentages not given if less than 10 observations incidence of such occurrences in relation to laboratory practice are considered the data must be interpreted carefully with reference to the particular patient groups concerned. In this study neisseriae were isolated from an anogenital site in 95 women (urethra, cervix, and rectum were tested routinely), but only one isolate (1%) proved to be a meningococcus. Since 3·2% of meningococcal isolates gave a false-positive reaction on initial testing a false-positive coagglutination result with a meningococcus from an anogenital site in a female patient is unlikely to occur in more than 1 in 3000 patients from whom anogenital neisseriae are isolated; this could be less than 1 in 30 000 patients investigated. We consider identification by immunological methods acceptable at the above levels particularly when it is borne in mind that colonial morphology may alert a trained observer to a misdiagnosis.

In homosexual men non-gonococcal neisseriae appear to be isolated from the anorectum more frequently. In this trial one of five neisserial isolates from the anorectum of men was a meningococcus. In an earlier study[17] we isolated *N meningitidis* from the anorectum of four (5·7%) of 70 homosexual men. We maintain our earlier view that it is important to confirm, preferably by a rapid carbohydrate utilisation test, the identity of neisseriae isolated from the anorectum of homosexual men. Tapsall and Cheng[18] in a recent comparative study also noted that a rapid test using preformed enzymes was superior both in terms of accuracy and rapidity than two other systems, both of which required growth of the organism to produce a positive result.

The Phadebact® gonococcus test in conjunction with culture on MNYC medium provides a very rapid and simple method of gonococcal identification for isolates from anogenital sites in women and from the urethra in men. Because technical manipulations are kept to a minimum the scheme lends itself well to large-scale screening for gonococcal infection among high-risk groups and quality control is easy for laboratories that isolate few gonococci.

We are grateful to our medical and non-medical colleagues in the departments of genitourinary medicine and bacteriology for their help with the collection and processing of specimens.

References

1. Danielsson D, Kronvall G. Slide agglutination method for the serological identification of *Neisseria gonorrhoeae* with anti-gonococcal antibodies adsorbed to protein A-containing staphylococci. *Appl Microbiol* 1974;**27**:368-74.
2. Barnham M, Glynn AA. Identification of clinical isolates of *Neisseria gonorrhoeae* by a coagglutination test. *J Clin Pathol* 1978;**31**:189-93.
3. Hampton KD, Stallings RA, Wasilauskas BL. Comparison of a slide coagglutination technique with the Minitek system for confirmation of *Neisseria gonorrhoeae. J Clin Microbiol* 1979;**10**:290-2.
4. Lewis JS, Martin JE. Evaluation of the Phadebact gonococcus test, a coagglutination procedure for confirmation of *Neisseria gonorrhoeae. J Clin Microbiol* 1980;**11**:153-6.
5. Lim DV, Wall T. Confirmatory identification of *Neisseria gonorrhoeae* by slide coagglutination. *Can J Microbiol* 1980;**26**:218-22.
6. Shanker S, Daley DA, Sorrell TC. A rapid slide coagglutination test—an alternative to the fluorescent antibody test for the identification of *Neisseria gonorrhoeae. J Clin Pathol* 1981;**34**:420-3.
7. Rufli T. Identification of *Neisseria gonorrhoeae* in the routine venereological laboratory. Comparative study of coagglutination, direct immunofluorescence, and sugar fermentation reaction. *Br J Vener Dis* 1980;**56**:144-7.
8. Young H. Identification and penicillinase testing of *Neisseria gonorrhoeae* from primary isolation cultures on Modified New York City medium. *J Clin Microbiol* 1978;**7**:247-50.
9. Young H, Harris AB, Urquhart, D, Robertson DHH. Screening by culture for the detection of gonorrhoea in women. *Scot Med J* 1979;**24**:302-6.
10. Svarva PL, Maeland JA. Comparison of two selective media in the cultural diagnosis of gonorrhoea. *Acta Pathol Microbiol Scand (B)* 1979;**87**:391-2.
11. Hookham AB. Thayer-Martin medium and modified New York City medium for the cultural diagnosis of gonorrhoea. *Br J Vener Dis* 1981;**57**:213.
12. Johnston NA. Evaluation of the coagglutination test for the identification of *Neisseria gonorrhoeae* in primary cultures. *Br J Vener Dis* 1981;**57**:315-9.
13. Futrovsky SL, Gaydos CA, Keiser J. Comparison of the Phadebact Gonococcus Test with the rapid fermentation method. *J Clin Microbiol* 1981;**14**:89-93.
14. Willmott FE. Meningococcal salpingitis. *Br J Vener Dis* 1976;**52**:182-3.
15. Miller MA, Millikin P, Griffin PS. *Neisseria meningitidis* urethritis. A case report. *JAMA* 1979;**242**:1656-7.
16. Givan KF, Thomas BW, Johnston AC. Isolation of *Neisseria meningitidis* from the urethra, cervix and anal canal. Further observations. *Br J Vener Dis* 1977;**53**:109-12.
17. McMillan A, Young H. Gonorrhoea in the homosexual man: frequency of infection by culture site. *Sex Transm Dis* 1978;**5**:146-50.
18. Tapsall JW, Cheng JK. Rapid identification of pathogenic species of *Neisseria* by carbohydrate degradation tests: importance of glucose in media used for preparation of inocula. *Br J Vener Dis* 1981;**57**:249-52.

Br J Vener Dis 1982;**58:**113-6

Epidemiological treatment of gonorrhoea and non-specific genital infection in female sexual contacts

Current practices in STD clinics in England and Wales

ELIZABETH M BELSEY

From the Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, London

SUMMARY In a study to quantify epidemiological treatment given to female contacts for gonorrhoea and non-specific genital infection in STD clinics in England and Wales two-thirds of women treated for gonorrhoea during 1978 were named contacts, a quarter of whom received epidemiological treatment. Only 35% of cases treated in this way were subsequently confirmed microbiologically, but nearly 1000 unconfirmed cases were returned as if they were true cases of gonorrhoea. In view of modern culture techniques and the low default rates found in this study, there seems to be little justification for the use of epidemiological treatment for gonorrhoea.

Of women who gave a history of exposure to non-specific urethritis, 86% received epidemiological treatment. The variation in diagnostic and notification criteria for non-specific genital infection results in virtually worthless statistics for female cases treated.

Introduction

Epidemiological treatment has been defined as treatment given to named contacts of patients after a history of exposure to disease but without or in advance of confirmatory pathological findings.[1] A survey of the diagnostic, treatment, and reporting criteria used in sexually transmitted diseases (STD) clinics in England and Wales in 1976 showed that epidemiological treatment would be given to female contacts of men with a diagnosis of gonorrhoea or non-specific urethritis in most clinics (85% and 76% respectively).[1, 2] In some contacts so treated for gonorrhoea, the diagnosis will be confirmed subsequently by culture. In 19% of clinics, however, consultants would return cases treated epidemiologically to the Department of Health and Social Security (DHSS) as 'real' cases of gonorrhoea even when the eventual smears and cultures gave negative results. In the remaining clinics such cases were returned as 'other conditions requiring treatment' (D2) or 'other conditions not requiring treatment' (D3). Cases treated epidemiologically for non-specific genital infection (NSGI) would be returned as 'true' cases in a high proportion (60%) of clinics. These findings imply over-reporting, not only of cases of gonorrhoea and NSGI but also of those in the diagnostic categories D2 and D3.

Little is known, however, about several factors which could affect the number of patients treated epidemiologically and thus the extent to which the published statistics are distorted. The above figures relate to clinics not to the numbers of patients seen in those clinics. The number of patients who visit clinics as named contacts is not known with any certainty. It was, therefore, decided that a study should be carried out to quantify epidemiological treatment of gonorrhoea and NSGI and to estimate the degree of over-reporting or misreporting, or both, that results. The study was limited to female contacts, since the previous study had shown that male contacts would be given epidemiological treatment in considerably fewer clinics than female.

Methods

A retrospective sample of female cases treated in STD clinics in England and Wales during 1978 was drawn in two stages, clinics being selected at the first stage and cases within selected clinics at the second. The sample design is fully described elsewhere.[3]

For cases diagnosed, treated, or returned as gonorrhoea during 1978 the following information was recorded: the patient's age and type of contact

Address for reprints: Miss E M Belsey, Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, London W1

Accepted for publication 11 September 1981

evidence provided (including none); dates of her first visit and the visit on which treatment was given; results of cervical, urethral, and rectal smears and cultures for each visit up to and including that on which the patient was treated; whether treatment given was epidemiological; and the diagnostic category used to return the case. Similar information was recorded for cases diagnosed, treated, or returned as NSGI, except that smear and culture results at each visit were replaced by the patient's symptoms and signs, the findings on microscopical examination of cervical and urethral smears (+, + +, or + + + leucocytes per high power field), and the results of other microbiological tests.

Results

EPIDEMIOLOGICAL TREATMENT FOR GONORRHOEA

A sample of 2933 cases diagnosed, treated, or returned as gonorrhoea was obtained. Exactly one-third of patients treated were not named contacts and thus could not have been given epidemiological treatment. Of the remaining patients who provided some evidence of a history of exposure to the disease, 24% were treated epidemiologically.

Among women treated in this way, 12% gave a verbal history of exposure to gonorrhoea. In another 2%, a verbal history was corroborated by staff at the clinic where the original male patient was treated. Forty-four per cent of female contacts attended the same clinic as the male patient, and the remaining 42% produced a contact slip. There was no evidence that the strength of the contact evidence provided influenced the physician's decision to give or withhold epidemiological treatment. Equally, the likelihood that a contact would receive this form of treatment was unrelated to her age.

Although the treatment given to a female contact may be epidemiological at the time, in that the results of microbiological tests obtained so far are negative, the diagnosis may be confirmed subsequently by smear in a few clinics where smear results are not immediately available or, more often, by culture. Thirty-five per cent of cases treated epidemiologically were later proved to be true cases of gonorrhoea.

Using the findings of this study, together with the number of female cases of gonorrhoea returned from all STD clinics in England and Wales during 1978 (21 529), it is estimated that in that year a total of 3665 (standard error 372) female cases were treated epidemiologically as if the patient had gonorrhoea. Microbiological confirmation of the diagnosis was obtained subsequently in 1278 of these cases. Such cases should properly be returned under the heading 'gonorrhoea' on the SBH60 form. In this study, however, interest centred on the way in which the cases treated epidemiologically, but never proved (2387), were returned. Forty-one per cent of these, or just under 1000 estimated cases, were returned as if they were true cases of gonorrhoea. These represent 5% of all female cases of gonorrhoea reported in 1978. Another 35% of unconfirmed cases were returned in the diagnostic category 'other conditions requiring treatment', while 7% were included with 'other conditions not requiring treatment', despite the fact that treatment had been given. Nine per cent (224) of cases were not reported at all. The remaining unconfirmed cases were returned either as 'non-specific genital infection' or in some other diagnostic category.

Six per cent of contacts who had negative smear results at their initial visit, were not treated epidemiologically, but were subsequently proved to be infected by culture defaulted before treatment. Among patients who attended with a contact slip, however, the default rate was only 2% compared with 14% among those who gave a verbal history of exposure to gonorrhoea.

EPIDEMIOLOGICAL TREATMENT FOR NSGI

A sample of 3265 cases diagnosed, treated, or returned as NSGI was obtained. Cases treated as NSGI fall into two main categories: those diagnosed by the physician on the basis of symptoms, signs, and microscopical findings; and those treated epidemiologically. This division is complicated by the fact that consultants in 40% of clinics do not recognise NSGI as a distinct clinical entity in women and therefore will not treat it as such.[4] All treatment given in these clinics must, by definition, be epidemiological. In addition, cultures for *Chlamydia trachomatis* or *Haemophilus vaginalis* are performed in a number of clinics. When the culture result is positive, the case must still be notified as NSGI, since no other more appropriate category is available on the SBH60.

Thirty-three per cent of patients treated for NSGI gave no history of exposure to non-specific urethritis. These patients were therefore treated because the physician recognised NSGI and diagnosed it as the primary condition. Fourteen per cent of patients who had attended the clinic as contacts were also treated only after the diagnosis had been made. A very high proportion of contacts (86%), however, received epidemiological treatment.

Among women given epidemiological treatment, 15% gave a verbal history of exposure to non-specific urethritis. In a further 1% a verbal history was confirmed by checking with the clinic at which the male patient had been treated. Twenty-five per cent of female contacts attended the same clinic as their partner, but the majority (59%) produced a contact slip. The proportion of contacts given epidemiological treatment did not differ according to the strength of the contact evidence provided or between age groups.

Only 2% of cases treated epidemiologically were subsequently proved by positive culture results for *Chlamydia trachomatis* or some other organism.

The number of female cases of NSGI returned from all STD clinics in England and Wales during 1978 (20 641), together with the findings of this study, may be used to estimate the number of cases treated epidemiologically and the numbers of such cases notified in the various diagnostic categories. Female cases treated epidemiologically as NSGI totalled 13 191 (standard error 1234). Culture confirmation was obtained subsequently for 286 of these cases. Over three-quarters (10 079) of the remaining 12 905 cases treated but never diagnosed were returned in the diagnostic category 'non-specific genital infection'. These cases comprised 49% of all female cases of NSGI notified in 1978. Thirteen per cent of unconfirmed epidemiologically treated cases (1737) were returned as 'other conditions requiring treatment'. No return was made for 464 (4%) cases.

Discussion

The aims of this study were simply to estimate the numbers of female contacts given epidemiological treatment for gonorrhoea or NSGI in one year and to quantify the over-reporting or misreporting that occurs as a result. Its purpose was not to make any judgments on the use of this form of treatment. In the light of the data collected, however, it is difficult to refrain from making some comments on the practice itself.

Twenty-four per cent of female contacts treated for gonorrhoea were treated epidemiologically, and nearly two-thirds of these cases remained unconfirmed. Recent studies have shown that over 90% of gonococcal infections are detected by the first set of tests.[5 6] It is, therefore, likely that most patients treated epidemiologically, in whom the diagnosis was not confirmed subsequently by culture, did not have gonorrhoea. It can be argued that at the time of treatment the physician is not to know whether a contact will prove to be infected or uninfected and is therefore justified in giving epidemological treatment. On the other hand, indiscriminate treatment has associated costs in addition to the obvious financial ones: the social cost to the patient who may feel stigmatised unnecessarily; and the clinical cost in terms of relative and total resistance of gonococci to antibiotics. In addition, infected contacts rarely default if not treated epidemiologically. Since only 2% of patients who presented with a contact slip failed to reattend for treatment, efficient contact tracing can apparently keep default rates to a minimum.

There is clearly no justification for the practice of returning unconfirmed epidemiologically treated cases as if they are true cases of gonorrhoea. Given the SBH60 as it stands, 'other conditions requiring treatment' is probably the most appropriate category. It has already been pointed out, however, that this diagnostic category is used as a dumping ground'.[7] Since epidemiological treatment will doubtless continue to be given, a separate heading for such cases is needed. At present, the number of cases of gonorrhoea notified nationally could increase or decrease simply because some consultants altered their treatment or notification policy.

The same considerations do not apply to the diagnosis, treatment, and notification of NSGI, since the diagnosis cannot be 'proved' as such. The variation in diagnostic and notification criteria between clinics, however, results in virtually worthless statistics for female cases treated. In some clinics all cases, whether diagnosed or treated epidemiologically, are returned under the heading 'non-specific genital infection'. In other clinics, where epidemiologically treated cases are returned as 'other conditions requiring treatment', and the consultant does not recognise or treat NSGI as a distinct clinical entity in women, it is not notified at all. The findings of this study further confirm the need for acceptable and reproducible diagnostic criteria for NSGI as a primary disease entity and for a separate category on the SBH60 in which cases treated epidemiologically could be returned. When more clinics have acquired the facilities to culture for *Chlamydia trachomatis*, another heading for such infections should also be provided.

I wish to thank Mrs Gillian Fenner and Miss Marcia Kelson who carried out much of the fieldwork. The study was supported as part of a research and development contract from the Medical Research Council.

References

1. Adler MW. Diagnostic, treatment, and reporting criteria for gonorrhoea in sexually transmitted disease clinics in England and Wales: 2 Treatment and reporting criteria. *Br J Vener Dis* 1978;54:15-23.
2. Adler MW. Diagnostic, treatment, and reporting criteria for non-specific genital infection in sexually transmitted disease clinics in England and Wales: 2 Treatment and reporting criteria. *Br J Vener Dis* 1978;54:428-32.

Br J Vener Dis 1982;**58**:117-20

Rosaramicin and tetracycline in the treatment of non-gonococcal urethritis

A comparison of clinical and microbiological results

J F DARNE,* G L RIDGWAY,† AND J D ORIEL*

*From the Departments of *Genitourinary Medicine and †Clinical Microbiology, University College Hospital, London*

SUMMARY The clinical and microbiological outcome of the treatment of 94 men for uncomplicated non-gonococcal urethritis (NGU) was studied. Rosaramicin 250 mg six hourly for seven days was given to 46 men and tetracycline 250 mg six hourly for seven days to 48 men; the follow-up period was up to six weeks. Complete resolution of the clinical signs of infection was seen in 40 (87%) of the men treated with rosaramicin and in 37 (77%) of those treated with tetracycline. *Chlamydia trachomatis* was eliminated from 17 of the 18 men treated with rosaramicin and from all of the 16 men treated with tetracycline. *Ureaplasma urealyticum* was eliminated from 12 of the 14 men treated with rosaramicin and from 15 of the 19 receiving tetracycline. Clinical recovery correlated well with the elimination of *C trachomatis* but less well with that of *U urealyticum*. The two antimicrobial agents were equally effective in the therapy of NGU, but gastrointestinal side effects were significantly more common in men treated with rosaramicin.

Introduction

Non-gonococcal urethritis (NGU) is one of the commonest sexually transmitted diseases. There is a substantial body of evidence from isolation studies, serology, and response to antimicrobial therapy to indicate that *Chlamydia trachomatis* causes 35-60% of cases.[1] The evidence implicating *Ureaplasma urealyticum* is less compelling, but this organism is believed to cause some cases.[2] At least one-quarter of NGU infections are of unknown aetiology.

Many years ago, empirical studies clearly showed that the tetracylines were clinically highly effective in the treatment of NGU.[3] These agents show in-vitro activity against *C trachomatis* and against many strains of *U urealyticum,* and studies of men with NGU treated with tetracyclines have linked clinical improvement with the eradication of one or both of these organisms.[4] Patients with NGU who harbour neither *C trachomatis* nor *U urealyticum* show the poorest clinical response to treatment.[5] The macrolide antibiotic, erythromycin, also gives good results in the treatment of NGU. Its in-vitro activity against *C trachomatis* is reflected by its efficacy in chlamydia-positive NGU, and it also gives good results in chlamydia-negative NGU.[6] It is active in vitro against many strains of *U urealyticum*, but its clinical efficacy against this organism has not been studied.

Rosaramicin is a macrolide antibiotic which is active against *Neisscria gonorrhoeae* and has given good clinical results in the treatment of gonorrhoea.[7] It is also active against both *C trachomatis* and *U urealyticum.* Good results have been obtained in the treatment of both chlamydia-positive and chlamydia-negative NGU[8]; its efficacy against *U urealyticum* associated with NGU has not yet been studied. In this investigation rosaramicin was compared with tetracycline in the treatment of NGU with regard to its clinical efficacy and action against *C trachomatis* and *U urealyticum.*

Patients and methods

CLINICAL PROCEDURES

A group of men with uncomplicated NGU was studied. The criteria for inclusion in the investigation were: (1) the presence of a visible urethral discharge; (2) a Gram stain of the discharge showing no intracellular diplococci and ≥5 polymorphonuclear leucocytes (PMNL) per high-power field at ×1000 magnification; (3) negative culture result of this material for *N*

Address for reprints: Dr J D Oriel, Department of Genitourinary Medicine, University College Hospital, Gower Street, London WC1E 6AU

Accepted for publication 18 September 1981

gonorrhoeae; (4) no antibiotic treatment for four weeks before enrolment; and (5) no clinical evidence of serious systemic disease nor history of allergy to tetracyclines or macrolides. Informed consent to the investigation was obtained from each patient.

At first attendance, a history was taken and the patient fully examined. Specimens were collected as follows: (1) urethral material was collected with a plastic loop (Nunc Products Ltd) and spread on a glass slide for Gram staining; (2) a second specimen was used to inoculate a culture plate for *N gonorrhoeae*; (3) an endourethral wire-mounted cottonwool swab (Medical Wire and Equipment Co) was passed 1-2 cm into the urethra, removed, and cut off into a tube of mycoplasma transport medium; (4) a second wire-mounted swab was passed 4-5 cm into the urethra, removed, and cut off into a tube of chlamydia transport medium; (5) in a two-glass urine test suspended material in the first glass was examined microscopically for PMNL and the midstream specimen examined for albumin and sugar and cultured for urinary pathogens; and (6) blood was collected for serological tests for syphilis, routine haematology, and blood chemistry.

TREATMENT ALLOCATION

After a diagnosis of NGU had been made the patients were assigned by random numbers to two treatment schedules: rosaramicin 250 mg six hrly for seven days or tetracycline 250 mg six hrly for seven days. Follow-up examinations were performed on all patients on the completion of treatment (day 7) and two weeks after (day 21); when possible, a third follow-up examination was performed four weeks later (day 49). On each occasion after clinical examination specimens (1) to (5) were examined as described above. Serological tests for syphilis, if initially giving negative results, were not repeated during the study, and haematological and biochemical tests were repeated only on day 7. Neither the patient nor the physician knew which drug had been taken, and the data were evaluated blindly. The two treatment groups were similar for demographic data and previous history of STD.

LABORATORY PROCEDURES

N gonorrhoeae was cultured on a selective medium.[9] Cell culture for *C trachomatis* was performed by centrifugation of specimens on to McCoy cells pretreated with idoxuridine followed by incubation and examination for chlamydial inclusion bodies.[10] A semi-quantitative technique modified from the method of Taylor-Robinson *et al* [11] was used for the culture of *U urealyticum*; tubes of liquid culture showing pH change were subcultured to solid media to confirm the presence of *U urealyticum*.

EVALUATION CRITERIA

Evaluation of the clinical response to therapy was performed after the completion of follow up and was classified as follows:

Complete resolution—disappearance of symptoms, no visible urethral discharge, <5 PMNL per field on microscopy of a Gram-stained urethral smear, and urine free from suspended matter;

Improvement—symptoms still present but no discharge, or symptoms absent but discharge still present although decreasing;

Treatment failure—no improvement in symptoms or signs and reinfection unlikely (no further sexual contact admitted);

Indeterminate—no improvement in symptoms or signs but reinfection possible, responses not falling into the other categories.

Microbiological evaluation was based on the results of culture for *C trachomatis* and *U urealyticum* before and after treatment.

STATISTICAL METHODS

The Mann-Whitney U test (two-tailed) was used to compare treatment differences for final clinical and microbiological evaluation. The Fisher exact test (two-tailed) was used for the assessment of side effects of therapy.

Results

A group of 137 men with NGU met the criteria for inclusion in the study but eight did not return after the first visit and a further 35 either failed to complete their follow-up examinations for at least 21 days or had incomplete laboratory data. Thus 94 men were studied fully; 46 were treated with rosaramicin and 48 with tetracycline.

CLINICAL RESPONSE

Complete resolution of the clinical signs of infection occurred in 40 (87%) of the 46 men treated with rosaramicin and in 37 (77%) of the 48 men treated with tetracycline (table I). These differences are not significant.

MICROBIOLOGICAL RESPONSE

Chlamydia trachomatis

C trachomatis was isolated from 18 of 46 (39%) men before treatment with rosaramicin (in 16 as the sole pathogen and in two in association with *U urealyticum*). Chlamydia were reisolated from one of these 18 patients during the follow-up period; he showed evidence of persistent urethritis and denied sexual intercourse since therapy. *C trachomatis* was isolated from 16 of 48 (33%) men before treatment

TABLE I *Clinical response of men with NGU treated with rosaramicin or tetracycline*

Treatment schedule	*No of patients*				
	Complete resolution of infection	*Incomplete resolution*	*Treatment failure*	*Indeterminate result*	*Total*
Rosaramicin 1 g daily for 7 days	40	4	2	0	46
Tetracycline 1 g daily for 7 days	37	6	2	3	48

with tetracycline (in nine as the sole pathogen and in seven in association with *U urealyticum*). Chlamydia were not reisolated from any of these patients during the follow-up period (table II). Differences in responses between those given rosaramicin and those given tetracycline were not significant.

TABLE II *Effect of treatment of non-gonococcal urethritis with rosaramicin or tetracycline on associated Chlamydia trachomatis and Ureaplasma urealyticum. (Figures in parentheses show numbers with complete clinical resolution)*

Isolation sequence	*No of patients treated with:*	
	Rosaramicin	*Tetracycline*
Chlamydia trachomatis		
Positive – positive	1 (0)	0
Positive – negative	17 (17)	16 (15)
Negative – positive	0	0
Negative – negative	28 (23)	32 (22)
Ureaplasma urealyticum		
Positive – positive	2 (1)	4 (3)
Positive – negative	12 (11)	15 (12)
Negative – positive	6 (5)	2 (2)
Negative – negative	26 (23)	27 (20)

Ureaplasma urealyticum

U urealyticum was isolated from 14 of 46 (30%) patients before treatment with rosaramicin and was reisolated from two of these patients during follow up; one showed signs of persistent urethritis but the other was clinically cured. In a further six patients *U urealyticum* was recovered during follow up although they had been isolation-negative before treatment; one showed persistent urethritis but the other five were clinically cured. *U urealyticum* was isolated from 19 of 48 (39%) men before treatment with tetracycline. It was reisolated from four of these patients during follow up; one showed persistent urethritis but three were clinically cured. In a further two patients *U urealyticum* was recovered during follow up although they had been isolation-negative before treatment; both were clinically cured (table II). Differences in responses between those given rosaramicin and those given tetracycline were not significant.

SIDE EFFECTS

No evidence of haematological or biochemical toxicity was noted in any patients treated with rosaramicin or tetracycline. In one man who was treated with rosaramicin liver function tests before treatment gave abnormal results. Total bilirubin was 39 µmol/l, conjugated bilirubin 4 µmol/l, alanine transaminase 94 IU/l, and y-glutamyl transferase 172 IU/l. Serum HBsAg was not present. A slight increase in these enzyme concentrations was noted after therapy; over the following six months they returned to within normal limits, although there was a persistent unconjugated bilirubinaemia. He probably had pre-existing Gilbert's disease with a mild attack of viral hepatitis unrelated to rosaramicin therapy.

Minor gastrointestinal side effects (nausea, diarrhoea, and abdominal discomfort) were described by 15 of the 46 (33%) men treated with rosaramicin; headache, dizziness, or fatigue were noted by three others. Gastrointestinal side effects were described by two of the 48 (4%) men treated with tetracycline; headaches or dizziness were noted by four others. The differences in gastrointestinal side effects between men treated with rosaramicin and tetracycline were significant ($P = 0 \cdot 01$).

Discussion

Thus, rosaramicin and tetracycline give similar results in the treatment of NGU, with no significant differences in the responses of *C trachomatis* or *U urealyticum* to either drug. Clinical recovery correlated well with the elimination of *C trachomatis* but less well with that of *U urealyticum*. There was a relatively high incidence of gastrointestinal side effects in men treated with rosaramicin which may limit its clinical use.

References

1. Schachter J. Chlamydial infections. *N Engl J Med* 1978; 298:428-35.
2. Taylor-Robinson D, McCormack WM. The genital mycoplasmas. *N Engl J Med* 1980; 302:1003-10.
3. Willcox RR. Triple tetracycline in the treatment of non-gonococcal urethritis in males. *Br J Vener Dis* 1972; 48:137-40.

4. Bowie WR, Yu JS, Fawcett A, Jones HD. Tetracycline in nongonoccoccal urethritis: comparison of 2 g and 1 g daily for seven days. *Br J Vener Dis* 1980; 56:332-6.
5. Bowie WR. Etiology and treatment of nongonococcal urethritis. *Sex Transm Dis* 1978; 5:27-33.
6. Oriel JD, Ridgway GL. Comparison of erythromycin stearate and oxytetracycline in the treatment of non-gonococcal urethritis: their efficacy against *C trachomatis. Scot Med J* 1977; 21:375-9.
7. Dacso C, Greenberg S, Martin RR. Rosaramicin treatment of gonococcal urethritis in men. *Sex Transm Dis* 1980; 7:133-4.
8. Juvakoski T, Allgulander C, Lassus A. Rosaramicin and tetracyline treatment in *C trachomatis*-positive and -negative nongonococcal urethritis. *Sex Transm Dis* 1981; 8:12-5.
9. Phillips I, Humphrey D, Middleton A, Nicol CS. Diagnosis of gonorrhoea by culture on a selective medium containing vancomycin, colistin, nystatin, and trimethoprim (VCNT). A comparison with Gram-staining and immunofluorescence. *Br J Vener Dis* 1972; 48:287-92.
10. Reeve P, Owen J, Oriel JD. Laboratory procedures for the isolation of *Chlamydia trachomatis* from the human genital tract. *J Clin Pathol* 1975; 28:910-4.
11. Taylor-Robinson D, Addey J, Goodwin CS. Comparison of techniques for the isolation of T-strain mycoplasmas. *Nature* 1969; 222:274-5.

Br J Vener Dis 1982;**58**:121-3

Metronidazole in a single dose for the treatment of trichomoniasis

Failure of a 1-g single dose

THOMAS W AUSTIN, ELIZABETH A SMITH, RIAD DARWISH, EDWARD D RALPH, AND FREDERICK L M PATTISON

From the Sexually Transmitted Diseases Clinic and the Family Planning Clinic, Middlesex-London District Health Unit; and the Department of Medicine, University of Western Ontario, London, Ontario, Canada

SUMMARY To determine the minimum effective dose of metronidazole in the treatment of vaginal trichomoniasis, a randomised clinical trial comparing single 1-g and 2-g doses was carried out on 163 patients attending sexually transmitted diseases and family planning clinics. Seventy-two of 86 (84%) patients receiving a single 2-g dose of metronidazole were cured compared with only 42 of 77 (55%) receiving a 1-g dose. The body weight of the patient was a significant variable affecting treatment outcome only in the latter group; 69% of patients weighing more than 57 kg or less were cured compared with only 43% of those weighing more. Patients who failed after either dose regimen were retreated with a single 2-g dose. Eighteen of 21 (86%) and seven of 10 (70%) failures with the initial 1-g and 2-g doses respectively were cured. A single 1-g dose of metronidazole is not recommended as routine treatment for vaginal trichomoniasis.

Introduction

A single 2-g dose of metronidazole is recognised as an effective treatment for trichomonal vaginitis and gives comparable results to the standard seven-day course of 250 mg three times daily.[1-11] The minimum single dose has not, however, been well established, although the efficacy of single 1-g and 1·5-g doses was reported by one investigator to be equivalent to a 2-g dose.[6 7] The purpose of this study was to confirm the preceding results with a 1-g dose in the hope of determining the lowest effective dose of metronidazole for routine use in trichomoniasis in order to minimise costs and any potential toxicity of the drug.

Patients and methods

From March 1978 to April 1980 a total of 186 female patients, 84 attending the sexually transmitted diseases clinic and 102 the family planning clinic at Middlesex-London District Health Unit, London, Ontario, Canada, were enrolled in a randomised single-blind prospective study, in which single 1-g and 2-g doses of metronidazole were compared. Patients were informed of the nature of the trial and follow-up procedures before entry into the study.

The diagnosis of trichomoniasis was initially suspected clinically in patients who complained of increased or malodorous vaginal discharge and perineal pruritus or soreness. This was confirmed by speculum examination, demonstration of viable trichomonads in a wet-mount preparation, and subsequent culture of the organism from vaginal secretions in modified Diamond's medium. After entry into the study, the patient's body weight and symptoms were recorded and she was randomly assigned to receive either a single 1-g or 2-g dose of metronidazole by a series of random numbers prepared by the pharamacy. The drug was dispensed as 250-mg tablets of Flagyl® (Rhone-Poulenc Pharma Inc, Montreal, Quebec, Canada) prepackaged in brown envelopes. The medication was taken in the presence of nursing staff but not the attending physician. Patients were told to abstain from alcoholic beverages for at least 48 hours. The principal sexual contact, if identified, was treated with a single 2-g dose of metronidazole. Patients were instructed to desist from sexual intercourse until their treatment response had been assessed.

For evaluation, a treatment cure was defined as a patient with a negative wet-mount examination and culture on the first return visit at least one week after

Address for reprints: Dr E D Ralph, Room 6-OF10, University Hospital, P O Box 5339, Stn A, London, Ontario, N6A 5A5 Canada

Accepted for publication 13 October 1981

treatment. The laboratory worker responsible for culturing the organism was not aware of the treatment given. After a treatment cure had been established, most patients were followed at subsequent clinic visits for longer than two weeks after treatment.

STATISTICAL ANALYSIS

χ^2 and Student's *t* tests were used.

Results

One hundred and sixty-three (88%) patients completed the study; 69 were followed in the sexually transmitted diseases clinic (STD) and 94 in the family planning clinic (FP). The age of the patients ranged from 15 to 50 years, with a mean of 22·7 years (23·1 years in the STD clinic and 21·9 years in the FP clinic patients). Mean body weights were also comparable in the two groups of patients (60·0 kg in the STD clinic and 59·1 kg in the FP clinic patients).

CURE RATES

Seventy-two of 86 (84%) patients were cured following a single 2-g dose of metronidazole compared with only 42 of 77 (55%) following a single 1-g dose (table I). The difference in cure rates was significant ($\chi^2_1 = 16{\cdot}46$, $P < 0{\cdot}001$).

TABLE I *Treatment results in patients with trichomoniasis attending the sexually transmitted disease (STD) or family planning (FP) clinic*

Dosage and group	*Treatment outcome*	
	Cure	*Failure*
1-g (n = 77)		
STD	21	12
FP	21	23
Total	42	35
2-g (n = 86)		
STD	31	5
FP	41	9
Total	72	14

The results of treatment for patients attending either the STD or FP clinic after each dose regimen are also shown in table I. After the 1-g dose, 21 of 33 (64%) of the STD patients were cured compared with only 21 of 44 (48%) of the patients attending the FP clinic. After the 2-g dose, 31 of 36 (86%) and 41 of 50 (82%) of the STD and FP clinic patients respectively were cured. Neither of these differences was significant ($\chi^2_1 = 1{\cdot}93$, $P > 0{\cdot}1$ and $\chi^2_1 = 0{\cdot}31$, $P > 0{\cdot}5$ respectively).

TIME OF CURE

The time of documentation of a treatment cure (first negative culture result) for the patients in both dosage groups is shown in table II. Approximately 60% of all patients who were cured had this diagnosis established on their return clinic visit one week after treatment. Of the remaining 40% of treatment cures, approximately half of the patients returned to the clinic either between one and two weeks or more than two weeks after treatment for reculture and confirmation of cure.

TABLE II *Time of documented cure and duration of follow up in patients after single 1-g and 2-g doses of metronidazole*

Dosage group	*No of patients cured (and followed up) at:*		
	1 week	*1-2 weeks*	*>2 weeks*
1g	25(7)	8(10)	9(25)
2g	39(4)	16(16)	17(52)

DURATION OF FOLLOW UP

Most patients were followed in subsequent clinic visits after their initial negative culture result. In both dosage groups, this follow-up period was longer than two weeks (table II).

SIDE EFFECTS

No severe side effects occurred in either group. Of the 77 patients receiving a 1-g dose, one complained of nausea and vomiting and four of nausea alone. Similarly, of the 86 patients receiving the 2-g dose, one complained of nausea and vomiting and six of nausea alone (one of whom admitted to concurrent alcohol ingestion). An eighth patient complained of an unpleasant taste in the mouth which was severe enough for her to refuse a second course of treatment.

RELATION TO BODY WEIGHT

When treatment outcome was related to body weight it was found to be a significant variable only for those treated with the 1-g dose. For this group, the mean weight of treatment cures was 57 kg (SD ± 9·1 kg) and of failures 63·7 kg (SD ± 13·3 kg) ($t = 2{\cdot}54$, degrees of freedom = 75, $P < 0{\cdot}02$). The lighter patients had a much better response although their cure rate of 69% was still less than that overall with the single 2-g dose (table III).

For the 2-g dosage group, the mean weights of treatment cures and failures were 58·9 kg (SD ± 11·1 kg) and 60·7 kg (SD ± 11·3 kg) respectively, which were not significantly different ($t = 0{\cdot}55$, degrees of freedom = 84, $P > 0{\cdot}05$).

TABLE III *Treatment results in two patient groups after a single 1-g dose of metronidazole*

Body weight (kg)	*Treatment outcome*		
	Cure	*Failure*	*Total*
≤57	24	11	35
>57	18	24	42

RETREATMENT

Treatment failures after either the 1-g or 2-g dose were retreated with a single 2-g dose of metronidazole. Of 21 patients who had failed initially with 1 g, 18 (86%) were cured compared with seven of 10 who had initially failed to respond to the 2-g dose.

Discussion

In a randomised prospective clinical study a single 2-g dose of metronidazole was shown to be significantly more effective than a 1-g dose. The treatment outcome in this study following the 2-g dose was similar to that reported previously in which cure rates ranged from approximately 80% to 95%.[1-4 7-11] The latter figure occurred in a clinic population where reinfection as a cause of treatment failure was recognised and apparently eliminated.[8] The proportion of treatment failures that were actually due to reinfection in our study, despite treatment of the principal male consorts and explicit instructions to the patients to avoid sexual intercourse, is unknown.

In the 1-g dosage group, the cure rate of only 55% is in clear contrast to the previously reported 100% cure rate.[6 7] The reason for this difference is not known but could be partly attributed to reinfection. As a result of randomisation, however, reinfection as a cause of treatment failure would be similar in both dosage groups and therefore should be no greater than 16% (the total failure rate after the 2-g dose). The conclusion from our study is that the single 1-g dose of metronidazole is significantly inferior to the 2-g dose and is not indicated for the routine treatment of symptomatic or clinically evident trichomoniasis.

Interestingly, the body weight of the patient was a significant variable affecting treatment outcome in the 1-g dosage group. To our knowledge, response to metronidazole treatment has not been correlated before with the patient's weight. In patients weighing 57 kg or less, 69% responded to the single 1-g dose but it was still inferior to the overall cure rate of 84% after a 2-g dose. Therefore, even in patients weighing less than 57 kg, a single 1-g dose could not be recommended routinely.

Optimal treatment after failure with a single 2-g dose is not well established. In our study, 70% of patients responded to a second 2-g dose which suggests that this is a reasonable therapeutic approach. Treatment failures after a second 2-g dose when reinfection is not a consideration should be investigated, however, for the possibility of a metronidazole-resistant strain of *Trichomonas vaginalis,*[12-14] malabsorption of the drug,[15] or excessive inactivation of metronidazole in the vaginal secretions by other organisms.[16 17]

It should be emphasised that all patients treated in this study were symptomatic or had clinically evident infection. Asymptomatic trichomoniasis diagnosed on routine vaginal cultures could possibly have a more favourable response to a single 1-g dose of metronidazole, although our study found no relationship between the severity of symptoms and response to treatment.

We are grateful to the staff of the sexually transmitted diseases and family planning clinics for their co-operation and help and to the Gastroenterology Research Unit, Victoria Hospital, for laboratory facilities. This study was funded by the Ontario Ministry of Health through Provincial Health Grant No PR786.

References

1. Csonka GW. Trichomonal vaginitis treated with one dose of metronidazole. *Br J Vener Dis* 1971;47:456-8.
2. Woodcock KR. Treatment of trichomonal vaginitis with a single oral dose of metronidazole. *Br J Vener Dis* 1972;48:65-8.
3. Morton RS. Metronidazole in the single-dose treatment of trichomoniasis in men and women. *Br J Vener Dis* 1972;48:525-7.
4. Ross SM. Single- and triple-dose treatment of *Trichomonas* infection of the vagina. *Br J Vener Dis* 1973;49:475-7.
5. Dykers JR jun. Single-dose metronidazole for trichomonal vaginitis. *N Engl J Med* 1975;293:23-4.
6. Dykers JR jun. Single-dose metronidazole for trichomonal vaginitis: a follow-up. *N Engl J Med* 1977;295:395-6.
7. Dykers JR jun. Single-dose treatment for trichomonal vaginitis: Patient and consort. *Am J Obstet Gynecol* 1978;132:579-80.
8. Fleury FJ, Van Bergen WS, Prentice RL, *et al.* Single dose of two grams of metronidazole for *Trichomonas vaginalis* infection. *Am J Obstet Gynecol* 1977;128:320-2.
9. Underhill RA, Peck JE. Causes of therapeutic failure after treatment of trichomonal vaginitis with metronidazole. Comparison of single-dose treatment with standard regimen. *Br J Clin Pract* 1974;28:134-6.
10. Thin RN, Symonds MAE, Booker R, *et al.* Double-blind comparison of a single dose and a five-day course of metronidazole in the treatment of trichomoniasis. *Br J Vener Dis* 1979;55:354-6.
11. Hager WD, Brown ST, Kraus SJ, *et al.* Metronidazole for vaginal trichomoniasis. *JAMA* 1980;244:1219-20.
12. Thurner J. Meingassner JG. Isolation of *Trichomonas vaginalis* resistant to metronidazole. *Lancet* 1978;ii:738.
13. Forsgren A, Forssman L. Metronidazole-resistant *Trichomonas vaginalis. Br J Vener Dis* 1979;55:351-3.
14. Heyworth R, Simpson D, McNeillage GJC, *et al.* Isolation of *Trichomonas vaginalis* resistant to metronidazole. *Lancet* 1980;ii:476-8
15. Roe FJC. Metronidazole: review of its uses and toxicity. *J Antimicrob Chemother* 1977;3:205-12.
16. Nicol CS, Evans AJ, McFadzean JA, *et al.* Inactivation of metronidazole. *Lancet* 1966;ii:441.
17. Ralph ED, Clarke DA. Inactivation of metronidazole by anaerobic and aerobic bacteria. *Antimicrob Agents Chemother* 1980;14:377-83.

Br J Vener Dis 1982;**58**:124-6

Treatment of vaginal candidosis with a single 500-mg clotrimazole pessary

IAN MILSOM AND LARS FORSSMAN

From the Department of Obstetrics and Gynaecology, East Hospital, Gothenburg, Sweden

SUMMARY In a double-blind study the therapeutic efficacy of a single 500-mg clotrimazole pessary was compared with that of a 200-mg clotrimazole pessary inserted once daily for three days in 72 patients with vaginal candidosis confirmed by culture. On clinical assessment four weeks after completion of treatment with the single-dose pessary the cure rate was 86% compared with 92% after the three-day regimen. There was no significant difference in the eradication rate between the single-dose (94%) and three-day regimens (89%). Four weeks after completion of treatment the recurrence rates by culture were 18% with the single-dose and 24% with the three-day regimen. The former treatment was well tolerated and as effective as the three-day clotrimazole regimen.

Introduction

Clotrimazole, one of the imidazole antimycotics, in a dosage of 100 mg given for six days was found to be effective in the treatment of vaginal candidosis.[1] Shorter periods of treatment of four[2] or three[3 4] days did not affect the efficacy of the treatment. Clotrimazole is fungicidal in vaginal secretions at a concentration of >10 μg/ml[5] and remains at this concentration for 72 hours after insertion of one 500-mg clotrimazole pessary (Ritter *et al*, unpublished data). We evaluated, therefore, the therapeutic efficacy and local tolerance of a single 500-mg clotrimazole pessary for the treatment of vaginal candidosis.

Patients and methods

Women with all the following criteria for vaginal candidosis were included in the study: (a) symptoms of irritation, burning, and vaginal discharge; (b) signs of inflammation of the vaginal mucosa and microscopical identification of pseudohyphae; and (c) cultural isolation of *Candida albicans.*

The culture specimens were promptly inoculated on to standard media and growing yeasts were identified as *C albicans* by the formation of chlamydospores and the germ tube test. Pregnant patients and those who had received antifungal or antitrichomonal treatment during the previous two weeks were excluded. Treatment with hormones or antibiotics, the use of an intrauterine contraceptive device, any previous treatment for vaginal candidosis, and the presence or absence of vulvitis (defined as irritation, swelling, and redness of the vulva) were recorded.

Cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* and microscopical examination to exclude *Trichomonas vaginalis* were performed before the patient started treatment.

TREATMENT

Patients were supplied with a plain numbered pack containing three pessaries marked day 1 to day 3. Each pack contained either one clotrimazole (500 mg) pessary plus two placebo pessaries or three clotrimazole (200 mg) pessaries. The active and placebo pessaries were indistinguishable. The containers were prepacked and randomised so that neither the clinician, the mycologist, nor the patient knew which treatment was being given. The first pessary was inserted at the completion of the gynaecological examination by one of us. The remaining pessaries were inserted once daily by the patient from the following night. Patients with vulvitis were also given clotrimazole cream and instructed to apply it to the vulva. No attempt was made to treat the sexual partner.

Address for reprints: Dr I Milsom, Department of Obstetrics and Gynaecology, East Hospital, S-416 85 Göteborg, Sweden

Accepted for publication 23 November 1981

FOLLOW UP

Patients were examined one and four weeks after the completion of treatment. At each follow up, the clinical symptoms were recorded using a three-point severity scale (0 = none, 1 = moderate, 2 = severe). A high vaginal swab was taken and examined with a light microscope for pseudohyphae and by culture for *Candida* species.

STATISTICAL ANALYSIS

The results of the two treatment regimens were compared using the χ^2 test with Yates's correction (significance, P<0·05).

Results

As treatment was started before mycological confirmation it was necessary to exclude seven women with negative culture results for *Candida* and one with positive culture results for both *C albicans* and *C trachomatis*. None of the patients had gonorrhoea as judged by culture samples from the cervix, urethra, and rectum. Seventy-two patients, ranging in age from 16-56 years, satisfied the criteria for inclusion and subsequently completed the trial. The characteristics of the two groups were similar (table I). The severity of clinical symptoms and signs are shown in table II.

TABLE I *Comparison of patients treated with either a single 500-mg clotrimazole pessary (group 1) or a 200-mg clotrimazole pessary for three days (group 2)*

	Group 1 (n = 35)	*Group 2 (n = 37)*
Age (years, mean ± SD)	27·4±7·6	29·0±6.9
Previously treated for vaginal candidosis	18	19
Vulvitis	33	35
Antibiotic treatment in month before trial	1	0
Contraceptive pill	15	15
Other hormone treatment (for example, oestrogen)	1	1
Intrauterine contraceptive device	8	8

n = No of patients treated; SD = standard deviation

CURE RATES

One week after completing treatment one patient in each of the two groups had signs and symptoms of vaginal candidosis (table III). They were retreated with 100-mg clotrimazole pessaries for six days. Four weeks later both patients were free of symptoms and culture results for *C albicans* were negative.

TABLE III *Patients with positive culture results for C albicans or signs and symptoms of infection one week and four weeks after completion of treatment*

	Results after completion of treatment (weeks):			
	Group 1 (n = 35)*		*Group 2* (n = 37)*	
	1	*4*	*1*	*4*
Positive culture result	2	6	4	8
Clinical signs and symptoms	1	5	1	3

* Group 1: treated with a single 500-mg clotrimazole pessary; group 2: treated with a 200-mg clotrimazole pessary for three days.

Four weeks after completing treatment five patients who had received a single 500-mg clotrimazole pessary and three the three-day regimen had signs and symptoms of vaginal candidosis (table III). This gave a cure rate of 85·7% four weeks after completion of the single-dose regimen and 91·9% for the patients given the three-day regimen. The difference in cure rates was not statistically significant ($\chi^2_1 = 0{\cdot}21$, P>0·05).

ERADICATION RATE

The eradication rate, based on a negative culture result for *C albicans*, seven days after treatment with a single clotrimazole pessary was 94·3% and that for the three-day regimen 89·2% (table III). The difference in eradication rates was not statistically significant ($\chi^2_1 = 0{\cdot}13$, P>0·05).

RECURRENCE RATE

Four weeks after completion of treatment the recurrence rate, as judged by culture, was 18·2% with

TABLE II *Total symptom score in the two treatment groups before and one and four weeks after completion of treatment*

	*Severity of symptoms**					
	Group 1† (n = 35)			*Group 2† (n = 37)*		
Symptoms	*Pretrial*	*1 week after*	*4 weeks after*	*Pretrial*	*1 week after*	*4 weeks after*
Itching	69	2	7	73	5	7
Burning	63	0	1	68	1	5
Discharge	59	1	1	61	3	4

* Severity scale: 0 = none, 1 = moderate, 2 = severe

† Group 1: treated with a single 500-mg clotrimazole pessary; group 2: treated with a 200-mg clotrimazole pessary for three days

n = No of patients treated

the single-dose regimen and 24·2% with the three-day regimen (table III). The difference in recurrence rates was not statistically significant ($\chi_1^2 = 0{\cdot}09$, $P > 0{\cdot}05$). None of the patients treated reported any side effects.

Discussion

Treatment of vaginal candidosis with a single 500-mg clotrimazole pessary was found to be as effective as previous treatment regimens of longer duration.[2 3 7] In the present investigation over 50% of the patients had been treated previously for vaginal candidosis, many several times each year. The duration and nature of the treatment is of special importance to women who have experienced repeated episodes of vaginal candidosis. Treatment with a single 500-mg clotrimazole pessary is an effective, simple, and acceptable regimen. The pessary can be inserted during the patient's clinic attendance as soon as the diagnosis is confirmed, which removes the problem of patient compliance and offers maintained efficacy and maximum acceptability.

References

1. Masterton G, Henderson J, Napier IR, Moffet M. Six-day clotrimazole therapy in vaginal candidiasis. *Curr Med Res Opin* 1975;**3**:83-8.
2. Milsom I, Forssman L. A double-blind trial of 4-days and 6-days therapy with clotrimazole in vaginal candidiasis. *Pharmatherapeutica* 1978;**2**:8-11.
3. Masterton G, Napier IR, Henderson JN, Roberts JE. Three-day clotrimazole treatment in candidal vulvovaginitis. *Br J Vener Dis* 1977;**53**:126-8.
4. Widholm O, Vartiainen E. Short-term clotrimazole treatment of vaginal candidosis. *Curr Ther Res* 1980;**28**:511-4.
5. Iwata K, Yamaguchi H, Hiratani T. Mode of action of clotrimazole. *Sabouraudia* 1973;**11**:158-66.
7. Highton BK. A trial of clotrimazole and nystatin in vaginal moniliasis. *Journal of Obstetrics and Gynaecology of the British Commonwealth* 1973;**80**:992-5.

Br J Vener Dis 1982;**58**:127-9

Balanoposthitis associated with Gardnerella vaginalis infection in men

G R KINGHORN,* B M JONES,† F H CHOWDHURY,* AND I GEARY†
*From the *Department of Genitourinary Medicine, Royal Infirmary, and the †Department of Medical Microbiology, University of Sheffield Medical School, Sheffield*

SUMMARY Fourteen of 194 (7·2%) consecutive unselected men had positive culture results from genital swabs for *Gardnerella vaginalis*. A higher yield of isolates was obtained from preputial (93%) than from urethral swabs (64%). Of the 14 men, two had no detectable genital abnormality, eight non-gonococcal urethritis, and nine balanoposthitis. The urethral isolation rates for *G vaginalis* in men with and without non-gonococcal urethritis were not significantly different, but preputial isolation rates were significantly higher ($p<0·001$) in men with balanoposthitis than in those without. The prevalence rate for *G vaginalis* in men with non-candidal balanoposthitis was 31%.

In a second study, concomitant *Bacteroides* species were isolated in preputial swabs from nine of 12 (75%) men with *G vaginalis*-associated balanoposthitis and may play a role in its pathogenesis.

Introduction

Gardnerella vaginalis (*Haemophilus vaginalis, Corynebacterium vaginale*) has been described as the most prevalent sexually transmitted organism in the causation of bacterial vaginitis.[1] Although women may harbour the organism without symptoms (as is the case with almost all genital pathogens), many infected women complain of an excessive malodorous vaginal discharge which is relieved when the organism is eradicated.

Evidence for the sexual transmission of *G vaginalis* rests on the higher prevalence rates in the more sexually active women attending sexually transmitted disease (STD) clinics[2] compared with those attending family planning[3] or gynaecological clinics and on the high isolation rates from the urethra[4-6] or urine[7] of men known to be consorts of women harbouring *G vaginalis*. Although *G vaginalis* has been isolated in men with non-gonococcal urethritis (NGU),[8 9] a causal role for the organism has not been established and asymptomatic colonisation of the male genitalia is assumed to be the rule.[10]

Previous work in this department[2] showed that *G vaginalis* was isolated from urethral, urine, or prostatic specimens in only a few men who were consorts of women infected with *G vaginalis*. In two men, however, much heavier growths of the organism were obtained from preputial swabs than from concomitant urethral swabs.

In this study we have investigated further the prevalence of *G vaginalis* in urethral and preputial swabs from a group of consecutive unselected men and attempted to determine whether the presence of *G vaginalis* on the male genitalia is associated with signs or symptoms of disease.

Patients and methods

One hundred and ninety-four consecutive unselected men presenting to the department of genitourinary medicine at this hospital with new complaints were prospectively studied at their first attendance. Urethral and preputial swabs (in circumcised men swabs were rolled on the glans penis and the coronal sulcus) were taken routinely, each immediately placed in peptone starch dextrose (PSD) transport medium, and left at room temperature until sent to the laboratory. This was usually done within an hour; the longest delay in transportation was three hours.

CULTURAL DIAGNOSIS

Swabs were inoculated on to Columbia blood agar plates[2] and incubated in 5% CO_2 at 37°C. After 48

Address for reprints: Dr G R Kinghorn, Department of Genitourinary Medicine, Royal Infirmary, Sheffield S6 3DA

Accepted for publication 19 November 1981

hours *G vaginalis* appeared as round raised glistening colonies, 0·5 mm in diameter, and often with a narrow zone of α-haemolysis. Gram-stained films of these colonies showed slender Gram-variable bacilli. Pure subcultures were confirmed by their inhibition by hydrogen peroxide, a negative catalase test result, and fermentation of glucose, maltose, starch, but not mannitol.

STATISTICAL ANALYSIS

The sites of isolation of *G vaginalis* and the clinical presentations in men with *G vaginalis* infections and in those without were compared by Yates's modified χ^2 test.

SECONDARY STUDY

In a second study we investigated symptomatic men and women with culture-confirmed *G vaginalis* infection for the presence of concomitant anaerobic bacteria using the cultural methods of Duerden *et al.*[11] The swabs taken for *G vaginalis* isolations were used for this.

Results

ISOLATION RATE

Of the 194 men in the initial prospective study, *G vaginalis* was isolated from the urethral or preputial swabs or both in 14, an isolation rate of 7·2%.

SITES OF ISOLATION

All 14 men were uncircumcised. The preputial swabs were the more productive; 13/14 (93%) of the preputial swabs gave positive results compared with nine (64%) of the urethral swabs.

CONCOMITANT GENITAL CONDITIONS

Only two men were without symptoms or signs of genital infection. Eight men had non-gonococcal urethritis, nine balanoposthitis, and one each genital herpes, genital warts, and pediculosis pubis.

Of 80 men presenting with NGU, *G vaginalis* was isolated from the urethra of six compared with three (2·5%) of 114 without NGU ($\chi^2_1 = 1 \cdot 5$, P>0·2).

Of 39 men with balanoposthitis, *G vaginalis* was isolated from the prepuce of nine compared with four of 155 men without balanoposthitis ($\chi^2_1 = 17 \cdot 8$, P<0·001). Yeasts were isolated from preputial swabs of 10 men with balanoposthitis, but in no patient were yeasts and *G vaginalis* isolated together. Thus, nine of 29 (31%) men with non-candidal balanoposthitis had *G vaginalis* isolated from their preputial swabs. No significant differences were found between men with balanoposthitis, with or without *G vaginalis* infections, and those without balanoposthitis in the prevalence of preputial isolations of commensal organisms such as *Staphylococcus albus*, diphtheroids, micrococci, or potential pathogens such as *Enterobacteriaceae, Staphylococcus aureus*, or streptococci.

CONCOMITANT ANAEROBIC INFECTIONS

In our second study, both *G vaginalis* and *Bacteroides* species were isolated from the same swabs from nine of 12 (75%) men with *G vaginalis*-associated balanoposthitis and from 22 (73%) of 30 women with *G vaginalis*-associated vaginitis. Such anaerobic infections may play a role in the pathogenesis of *G vaginalis*-associated conditions.

G VAGINALIS-ASSOCIATED BALANOPOSTHITIS

Clinical features

A total of 21 men with balanoposthitis associated with the presence of *G vaginalis* were seen in the two studies. The onset of symptoms occurred in all men within seven days of their most recent sexual contact, most often within two days. They consisted of a mild irritation of the prepuce and glans penis usually associated with an excessive offensive-smelling subpreputial discharge. Men with an acquired or congenital phimosis or long redundant prepuces in whom hygiene was deficient were particularly affected. After retraction of the prepuce and removal of the mucoid discharge erythematous non-erosive macules or papules were visible on the mucosal aspect of the prepuce and the glans penis.

Discussion

Our prevalence rate of 7·2% for *G vaginalis* isolation in unselected men was similar to that reported by Karpovaskaya[13] (8%). The study confirms an earlier impression[2] that in uncircumcised men *G vaginalis* more frequently colonises, or is easier to isolate from, the glans penis and prepuce than from the urethra, where the organism can easily be eradicated by micturition. We found no evidence to implicate the organism as a cause of non-gonococcal urethritis, but our results suggest that subpreputial carriage of *G vaginalis* is associated with balanoposthitis in some men, particularly those with a relative phimosis. Typically, the condition is mild with preputial irritation and an offensive fishy odour and is associated with signs of increased subpreputial mucoid discharge and mild inflammation of the glans penis and prepuce.

Further study is necessary to determine the pathogenesis of this condition. Chen *et al*[13] have postulated that in non-specific vaginitis a symbiotic relationship between *G vaginalis* and other organisms, particularly anaerobic bacteria, results in the production of amines which produce the characteristic symptoms. The frequent association of *G vaginalis* with *Bacteroides* species in subpreputial swabs from men with balanoposthitis suggests that a similar mechanism might result in the typical fishy smell and increased subpreputial secretion.

We thank Mr W E Shaw and his nursing staff for their generous co-operation with this study, and also Mrs C Treweek and Mrs E Welch for typing the manuscript.

References

1. Dunkelburg WE. *Corynebacterium vaginale*. A review. *Sex Transm Dis* 1977;**4**:69-75.
2. Bhattacharyya MN, Jones BM. *Haemophilus vaginalis* infection—its diagnosis and treatment. *J Reprod Med* 1980;**24**:71-5.
3. Bramley HM, Dixon RA, Jones BM. *Haemophilus vaginalis* (*Corynebacterium vaginale; Gardnerella vaginalis*) in a family planning clinic population. *Br J Vener Dis* 1981;**57**:62-6.
4. Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis. *Am J Obstet Gynecol* 1955;**69**:962-76.
5. De La Fuente F, Rico LR, Soria F. Hemofilasis Urogenital Afection Venerea? *Rev Esp Obstet Ginecol* 1959;**18**:252-4.
6. Pheifer TA, Forsyth PS, Durfee MA, Pollock HM, Holmes KK. Non-specific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. *N Engl J Med* 1978;**298**:1429-33.
7. Leopold S. Heretofore undescribed organism isolated from the genito-urinary system. *US Armed Forces Medical Journal* 1953;**4**:263-6.
8. Dunkelberg WE, Woolvin SC. *Haemophilus vaginalis* relative to gonorrhoea and male urethritis. *Milit Med* 1963;**128**:1098-101.
9. Sylvestre L, Ethier J. Treatment of urethritis in males with oxytetracycline. *Can Med Assoc J* 1963;**89**:1218-20.
10. Gardner HL. *Haemophilus vaginalis* after 25 years. *Am J Obstet Gynecol* 1980;**137**:385-91.
11. Duerden BI, Holdbrook WP, Collee JG, Watt B. The characterisation of clinically important Gram-negative anaerobic bacilli by conventional bacteriological tests. *J Appl Bacteriol* 1976;**40**:163-88.
12. Karpovaskaya O. The role of *Haemophilus vaginalis* infection of the female genitalia. *Akush Ginekol (Mosk)* 1971;**47**:33.
13. Chen KCS, Forsyth PS, Buchanan TM, Holmes KK. Amine content of vaginal fluid from untreated and treated patients with non-specific vaginitis. *J Clin Invest* 1979;**63**:828-35.

Br J Vener Dis 1982;**58:**130-3

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Lack of effect of bicarbonate on the survival of *Treponema pallidum* (Nichols) in vitro

Sir,
No published studies have reported the effect of bicarbonate (HCO_3^-) on the survival of *Treponema pallidum* in vitro.

Carbon dioxide (CO_2) has been shown to be beneficial to *Neisseria gonorrhoeae, N meningitidis,* some stapylococci,[1] and other bacteria.[2 3] CO_2 serves as a carboxylating agent[4] and as an essential precursor for the synthesis of certain amino acids.[5] It is also required for fatty acid synthesis,[1 6] biosynthesis of purine ribonucleotides, which are precursors of DNA and RNA and are an integral part of many coenzymes.[6]

Different concentrations of sodium bicarbonate ($NaHCO_3$) were used in both a cell-free and cell-culture system to determine whether bicarbonate had any beneficial effect on the survival of *T pallidum* in vitro.

The medium in the cell-free system[7] was slightly modified by replacing the sodium thioglycollate with 2 mmol/l dithiothreitol (DTT). In the cell-culture system, Eagle's minimal essential medium with 10% heat-inactivated fetal calf serum, 0·5 mmol/l DTT, and 20 mmol/l N-2-hydroxyethylpiperazine-N-2-ethane-sulphonic-acid (HEPES) was used.

T pallidum was harvested from infected rabbit testes.[7] Anaerobic incubation has been described.[7] For microaerophilic incubation, tubes with cottonwool plugs were equilibrated with 3% O_2[7] for two days before inoculation with treponemes. Treponemes were then added to an approximate final density of 5×10^6/ml, followed by HCO_3^- to give a final concentration of from 10^{-5} to 10^{-2} mol/l. The cottonwool plugs were immediately changed to rubber stoppers to prevent escape of CO_2. The pH remained at 7·3±0·2 in the cell-free system. The incubation temperature was 34°C.

The isolation and subculture of the rabbit testicular cell lines have been described.[8]

Viability of *T pallidum* in vitro was determined by the percentage motility under darkfield microscopy and their virulence in rabbits.[7] In the cell-culture system, attached treponemes were separated from tissue cells[8] before virulence was tested.

Ten experiments were performed in a cell-free medium under anaerobic conditions; a representative experiment is shown in the figure. There was no enhancement by HCO_3^- (10^{-2} to 10^{-5} mol/l) on the retention of motility of *T pallidum* (figure; *a*); the percentage motility in the presence of 10^{-3} and 10^{-2} mol/l HCO_3^- was consistently lower than the control. Similarly, HCO_3^- (10^{-2} to 10^{-5} mol/l) had no beneficial effect on the retention of virulence of *T pallidum* in vitro (figure; *b*).

Bicarbonate (10^{-5} to 10^{-2} mol/l) showed no enhancement of treponemal survival in two experiments under microaerophilic conditions with 3% O_2. The percentage motility (figure; *c*) and latent periods (figure; *d*) were virtually the same at all concentrations tested and were not appreciably different from the control tubes lacking bicarbonate.

In three experiments in cell-cultures, the pH of the medium dropped to 6·8 at day 4 in the control tubes while remaining at 7·2 in the presence of HCO_3^-/CO_2. When the pH of the medium was regularly adjusted, however, HCO_3^- (10^{-4} to 10^{-2} mol/l) had no enhancing effect on the retention of treponemal motility (figure, *e*) or virulence (figure, *f*) in the presence of tissue culture cells. Thus, in the media tested, HCO_3^-/CO_2 may serve mainly as a buffering system rather than as a reactant in any essential metabolic process.

Possible reasons why HCO_3^-/CO_2 had no enhancing effect on treponemal survival in vitro may be: (a) CO_2 requirements were satisfied by its endogenous metabolism, such as degradation of pyruvate and glucose.[9 10] (b) *T pallidum* under conditions which do not allow multiplication may have no capacity to use CO_2 even if it normally utilises it. (c) Even if *T pallidum* had the capacity to incorporate CO_2, it may be unnecessary. Since the medium was extremely rich, the presence of these nutrients may have shut down certain biosynthetic pathways. The incorporation of $NaH^{14}CO_3$ was insignificant when compared to total glucose incorporated.[10] (d) CO_2 would not be used for fatty acid synthesis since *T pallidum,* like other treponemes, cannot synthesise fatty acids.[11] (e) HCO_3^-/CO_2 may serve as a buffer in a closed system, but with HEPES (20 mmol/l) serving this purpose HCO_3^-/CO_2 was unnecessary in both the cell-free and cell-culture systems.

We gratefully acknowledge the excellent technical assistance provided by Mrs L Drummond and thank Professor S Faine and Dr Ben Adler for constructive critical evaluation. This work was supported by grants from Australian National Health and Medical Research Council (NR & MRC); the Utah Foundation, the Heiser Program, and Monash University.

Yours faithfully,
G H W Wong
B M Steiner
S R Graves

Department of Microbiology,
Monash University,
Victoria,
Australia.

References

1. Davis BD, Dulbecco R, Eisen HN, Ginsberg HS. *Microbiology.* Harper International Edition (3rd edition). Maryland, USA: Harper & Row, 1980.
2. Mickelson MN. Chemically defined medium for growth of *Streptococcus pyogenes. J Bacteriol* 1964;**88:**158-64.
3. Lachina RVF, Hartman PA. Carbon dioxide fixation and the synthesis of aspartic acid by *Streptococcus faeclum* var *durans. Biochem Biophys Res Commun* 1968;**32:**691-5.
4. Lyman CM, Mosely O, Wood S, Butter B, Hale F. Some chemical factors which influence amino acid requirements of lactic acid bacteria. *J Biol Chem* 1947;**167:**177-87.
5. Hancock R, McManus F. Carbon dioxide fixation in the synthesis of aspartic acid by a strain of *Staphylococcus aureus. Biochim Biophys Acta* 1960;**42:**152-4.
6. Lehninger AL. *Biochemistry,* 2nd ed. New York: Worth, 1976.
7. Steiner B, Mclean I, Graves S. Redox potential and survival of virulent *Treponema pallidum* under microaerophilic conditions. *Br J Vener Dis* 1981;**57:**295-301.
8. Fitzgerald TJ, Miller JN, Sykes JA. *Treponema pallidum* (Nichols strain) in tissue cultures: cellular attachment, entry, and survival. *Infect Immun* 1975;**11:** 1133-40.
9. Barbieri JT, Cox CD. Pyruvate oxidation by *Treponema pallidum. Infect Immun* 1979; **25:**157-63.
10. Barbieri JT, Cox CD. Glucose incorporation by *Treponema pallidum. Infect Immun* 1979;**24:**291-3.
11. Smibert RM. Spirochaetales, a review. *CRC Crit Rev Microbiol* 1973;**2:**491-552.

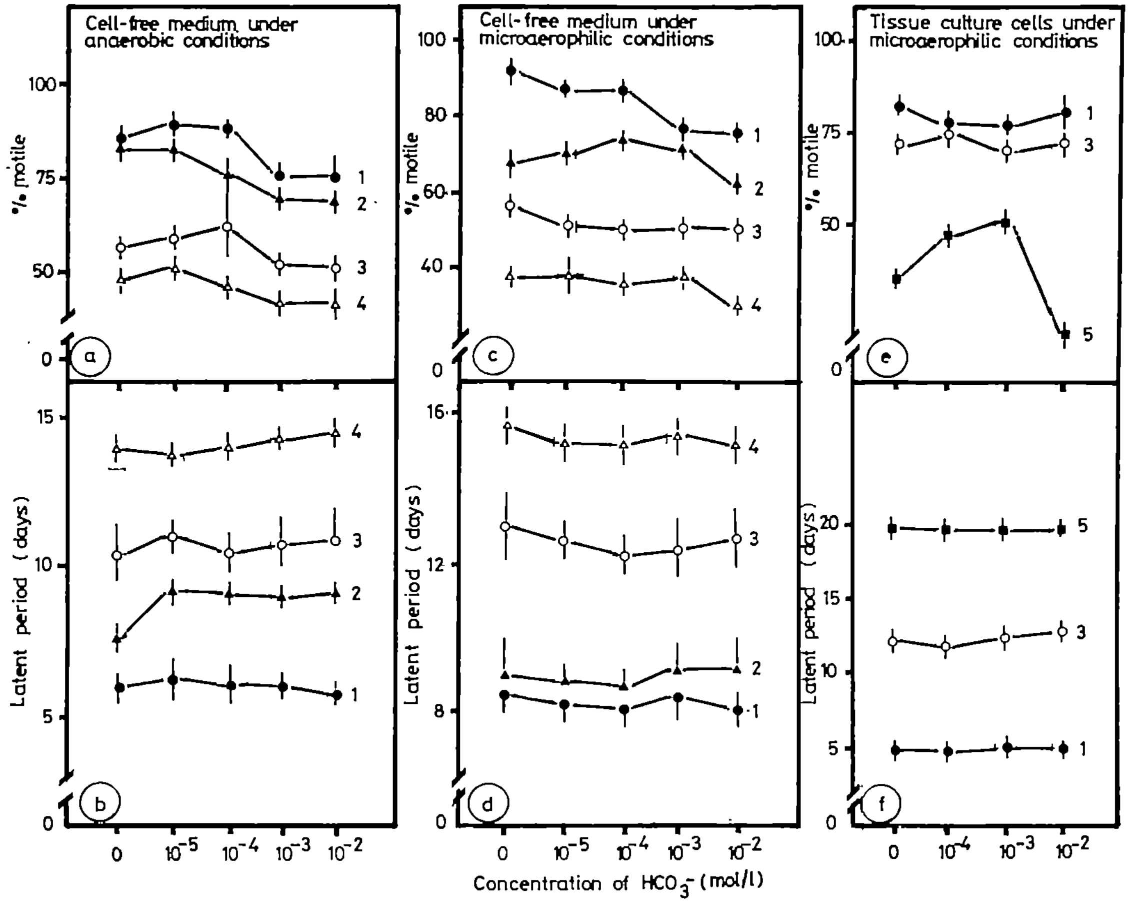


FIGURE *The effect of different concentrations of sodium bicarbonate on the survival of T pallidum in different cultures. (a) Percentage motility, mean ± SD of duplicate or triplicate samples; (b) latent period (days) for lesion development in rabbits, mean of 12 lesions in two rabbits ± SD; (c) percentage motility, mean ± SD of duplicate or triplicate samples; (d) latent period (days) for lesion development in rabbits, mean ± SD of six lesions in one rabbit; (e) percentage motility, mean of duplicate samples ± SD; and (f) latent period (days ± SD), mean of six lesions in one rabbit. (Numbers on the graphs denote time (days) at which T pallidum culture was examined.)*

TO THE EDITOR, *British Journal of Venereal Diseases*

Adenovirus type 19 and a closely related new serotype in genital infections

Sir,

In a report from this laboratory[1] the prevalence of adenovirus type 19 in infections of both male and female genital tracts has been noted in Western Australia. De Jong and associates have also reported the isolation of adenoviruses from the genital tract.[2] These were originally thought to represent hybrids of types 10, 13, 19, and 30,[3] but later work showed them to be sufficiently distinct both antigenically[2] and on the molecular level[4] to be considered a new serotype.

The prototype strain has been submitted as candidate adenovirus type 37.[2] After an exchange of virus strains and antisera between ourselves and de Jong's laboratory, standard neutralisation tests clearly indicated that some isolates from Western Australia previously regarded as adenovirus 19 were most similar to de Jong's new prototype virus (hereafter referred to as adenovirus 37 for convenience).

Of a selection of 26 genital and ocular adenoviruses isolated in these laboratories in 1977 and 1981 two were adenovirus 19 and 24 adenovirus 37. Two of the adenovirus 37 typings were from a cervical swab and an eye swab taken 18 days apart from the same patient. Rabbit antiserum raised against one of our isolates neutralised prototype adenovirus 37 but not prototype adenovirus 19 (obtained from Fairfield Hospital, Fairfield, Victoria) when 20 doses of antiserum were tested against 100 infectious doses of virus. A close antigenic relationship was found to exist, however, between adenovirus 19 and 37, leading to confusion in identification of the latter serotype when, for convenience, neutralisation tests were set up with 20 neutralising doses of antiserum against untitrated newly isolated virus.

The results shown in the table clearly indicate that both adenovirus 19 and 37 infect the human genital tract. We have confirmed the evidence obtained by de Jong *et al*[2] for the occurrence of adenovirus 37 in the eye and genital tract. These findings represent the first report of isolation of adenovirus 37 in the southern hemisphere (de Jong, personal communication). Extrapolation from these results to those we reported previously[1] would suggest that about 8% of isolates were adenovirus 19 and approximately 92% adenovirus 37. The association of human genital adenovirus infections with types 19 and 37[1 2 4-6] has been extended by other unpublished results from this laboratory showing that adenovirus 8, 9, 10, and 26 can also be isolated from genital specimens. It should be noted that all genital isolations of adenoviruses 8, 9, 10, and 26 in this laboratory and the two adenovirus 19s referred to in the table are neutralised only by the homologous WHO specific antiserum and not by antiserum to prototype adenovirus 37.

TABLE *Results of neutralisation tests on 26 isolates previously reported as adenovirus type 19*

Neutralising antiserum	*Urethral swab*	*Cervical swab*	*Eye swab from STD patients*
Adenovirus 19	2	0	0
Adenovirus 37	13	8	3

All the above adenoviruses belong to the human erythrocyte-agglutinating subgroups[7] of Rosen's group II[8] adenoviruses. It would be of interest to determine whether other members of these subgroups have a similar predilection for the mucous membranes of the genital tract and the eye. In the near future we will be reporting in more detail our laboratory and clinical findings in human genital adenovirus infections.

This letter has been published with permission from Dr J C McNulty, Commissioner of Public Health, Western Australia.

Yours faithfully,

P A Phillips
G B Harnett
M M Gollow

State Health Laboratory Services,
Queen Elizabeth II Medical Centre,
Nedlands,
Western Australia 6009

References

1. Harnett GB, Newnham WA. Isolation of adenovirus type 19 from the male and female genital tracts. *Br J Vener Dis* 1981;57:55-7.
2. de Jong JC, Wigand R, Wadell G, *et al.* Adenovirus 37: identification and characterisation of a medically important new adenovirus type of subgroup D. *J Med Virol* 1981; 7:105-18.
3. Schaap GJP, de Jong JC, van Bijsterveld OP, Beekhuis WH. A new intermediate adenovirus type causing conjunctivitis. *Arch Ophthalmol* 1979;97:2336-8.
4. Wadell G, Sundell G, de Jong JC. Characterisation of candidate adenovirus 37 by SDS-polyacrylamide gel electrophoresis of virion polypeptides and DNA restriction site mapping. *J Med Virol* 1981;7:119-25.
5. Laverty CR, Russel P, Black J, Kappagoda N, Benn MG, Booth N. Adenovirus infection of the cervix. *Acta Cytol (Baltimore)* 1977;21:114-7.
6. Cevenini R, Donati M, Landini MP, la Placa M. Adenovirus associated with an oculogenital infection. *Microbiologica* 1979;2: 425-7.
7. Hierholzer JC. Further subgrouping of the human adenoviruses by differential agglutination. *J Infect Dis* 1973;128:541-50.
8. Rosen L. A haemagglutination-inhibition technique for typing adenoviruses. *Am J Hyg* 1960;71:120-8.

TO THE EDITOR, *British Journal of Venereal Diseases*

An outbreak of gonorrhoea due to penicillinase-producing *Neisseria gonorrhoeae* (PPNG) in a provincial city

Sir,

Between October 1980 and July 1981 52 patients (31 men and 21 women) with gonorrhoea due to penicillinase-producing *Neisseria gonorrhoeae* (PPNG) attended the clinic at Cardiff Royal Infirmary. During the previous 12 months only three such infections were recorded.

Patients with uncomplicated gonorrhoea whose secretions contained Gram-negative diplococci were treated routinely with ampicillin 2 g and probenecid 1 g by mouth. Cultures were performed on chocolate agar and modified New York City medium.[1] Tests for sensitivity to penicillin and penicillinase production[2 3] were carried out on all isolates. When infection by PPNG was established, patients were retreated with a single injection of 2 g spectinomycin. Four women infected with PPNG had complications (pelvic infection three and Bartholin's abscess one); they were treated with 1 g kanamycin daily for three days. Four men with PPNG infections developed epididymo-orchitis and were given co-trimoxazole two tablets twice daily for seven days. One man had epididymitis at the time of his first visit, the other seven patients developed their complications within a week of their initial treatment with ampicillin and probenecid. During the period reviewed seven of 209 men and nine of 137 women infected with non-PPNG strains developed complications. The difference in incidence of complications due to infections with PPNG and non-PPNG strains is significant ($\chi^2_1 = 7{\cdot}435$, $P<0{\cdot}01$). It was noted during the outbreak that the PPNG strains were gradually becoming resistant to other antibiotics.

Most of the patients infected with PPNG (86%) were Caucasians; the remainder were foreigners living in Wales.[4] One patient was infected in Bangkok, the other infections were contracted in the United Kingdom, and 60% of the men were infected by four Cardiff prostitutes. Fifty-two per cent of the patients were from the middle social classes, 16% came from a socially deprived background, and 32% were unemployed.

Yours faithfully,

L Cohen
S K Panja

Department of Genitourinary Medicine,
Cardiff Royal Infirmary,
Cardiff.

References

1. Young H. Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium. *Br J Vener Dis* 1978;54:36-40.
2. Phillips I. Beta-lactamase-producing penicillin-resistant gonococcus. *Lancet* 1976;ii:656-7.
3. Phillips CW, Aller RC, Cohen CN. Penicillinase-producing *Neisseria gonorrhoeae. Lancet* 1976;ii:960.
4. Arya OP, Rees E, Percival A, Alergant CD, Annels EH, Turner GC. Epidemiology and treatment of gonorrhoea caused by penicillinase-producing strain in Liverpool. *Br J Vener Dis* 1978;54:28-35.

TO THE EDITOR, *British Journal of Venereal Diseases*

Isolation of *Haemophilus ducreyi* from the conjunctiva

Sir,

The report by Gregory *et al* of conjunctivitis due to *Haemophilus ducreyi*[1] was most interesting. However, the authors have not provided sufficient information to confirm that the isolate was in fact *H ducreyi.* In our experience[2] the appearance of macroscopic colonial growth after only 24 hours is unusual for *H ducreyi,* and the fact that the organism required X factor but not V factor for growth does not distinguish *H ducreyi* from *H aphrophilus* or *H haemoglobinophilus.*[3 4] All three of these organisms usually reduce nitrate to nitrite, although exceptions occur; failure of the isolated organism to do so is not definitive.[3] A porphyrin test (incubation of the organism with delta-aminolevulinic acid) and a catalase test would have been helpful; *H ducreyi* gives negative results with both tests, *H aphrophilus* is porphyrin-positive and catalase-negative, and *H haemoglobinophilus* is catalase-positive and porphyrin-negative.[3 4]

Hopefully, the authors will have saved their organism in order to perform these tests or to send it to a reference laboratory for definitive identification.

Yours faithfully,

*H H Handsfield**
C L Fennell†

*Seattle-King County Department of Public Health, and
†Department of Medicine, Harborview Medical Center and the University of Washington,
Seattle,
Washington, USA

References

1. Gregory JE, Henderson RW, Smith R. Conjunctivitis due to *Haemophilus ducreyi* infection. *Br J Vener Dis* 1980;**56**:414.
2. Handsfield HH, Totten PA, Fennell CL, Falkow S, Holmes KK. Molecular epidemiology of *Haemophilus ducreyi* infections. *Ann Intern Med* 1981;**95**:315-8.
3. Kilian M. A taxonomic study of the genus *Haemophilus,* with the proposal of a new species. *J Gen Microbiol* 1976;**93**:9-62.
4. Sottnek FO, Biddle JW, Kraus SJ, Weaver RF, Stewart JA. Isolation and identification of *Haemophilus ducreyi* in a clinical study. *J Clin Microbiol* 1980;**12**:170-4.

Book review

Actualités Physio-biologiques sur la Syphilis: Monographie, Semaine des Hôpitaux. By P Collart and M Poitevin, 1981. Société d'Edition de l'Association d'Enseignment Médicale des Hôpitaux de Paris, Paris, France. (Obtainable from Institut Alfred Fournier, Paris). Pp 52. Price: frs 45·00.

This monograph is a collection of four reviews of selected aspects of research on syphilis which have appeared in *Semaine des Hôpitaux.* The aim of the authors is to relate the results of basic research to clinical and serological findings.

The first part deals with the basic bacteriology of *Treponema pallidum,* its morphology, method of reproduction, and antigenic structure. The second describes the evolution of the disease in experimental animals and contrasts this with the course of infection in man. The behaviour of *T pallidum* in cell-culture systems is not described, although this is perhaps the most rapidly advancing field of research at present. Problems of immunity are considered in the third section and the humoral and cellular aspects described.

In the discussion of serological tests as a reflection of the immune response, a more detailed appraisal of the possible importance of the various immunoglobulin classes of antitreponemal antibody would have been of interest. The final section is devoted to treatment with penicillin in the light of present knowledge of the pathology of syphilis. A single uniform treatment is not thought to be applicable, and in selecting the form to be used factors such as the stage of disease, type of preparation, and the age and weight of the patient must be considered.

The French school, led by Professor Collart, has made many notable contributions to our knowledge of syphilis. This monograph can be recommended to all those seeking a succinct account of its views. The wealth of references (483) also makes it a very useful guide to the literature on the topics reviewed.

A E Wilkinson

Br J Vener Dis 1982;**58:**134-8

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea
(Clinical; microbiology; therapy)
Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases
Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (clinical and therapy)

Neurosyphilis (editorial)
Br Med J 1981;**283:**263.

Secondary syphilitic uveitis
MW BELIN, AC BALTCH, AND PB HAY (Albany Medical College, Albany, New York, USA) *Am J Ophthalmol* 1981;**92:**210-14.

A case of secondary syphilis occurred in a 61-year-old-man who had presented with a panuveitis in both eyes which was unresponsive to steroids and associated with visual deterioration. There was no previous history of primary infection. The palms of the hands showed the erythematous macules of secondary syphilis. The lymphocyte count in the cerebrospinal fluid was increased. Fundoscopic examination showed macular oedema and slit-lamp examination keratitic precipitates with a flare in the aqueous humour. After aspiration of the vitreous and aqueous humours treponemes consistent with *T pallidum* were detected by darkground illumination with fluorescent antibody staining (although exact details of the technique used are not given) in the aqueous only.

Treatment with topical and systemic prednisolone (80 mg/day) was started before administration of intravenous penicillin G 3·5 megaunits every four hours. Despite taking prednisolone, the patient had a severe Jarisch-Herxheimer reaction with considerable loss of visual acuity in the unoperated eye, which slowly improved during the following week. After 11 days the uveitis had cleared. An anterior chamber paracentesis after a further eight weeks showed no treponemes. Visual acuity was nearly normal.

The authors' illustrations are very clear.

G D Morrison

Acute syphilitic optic neuritis
JM WEINSTEIN, SS LEXOW, P HO, AND A SPECKARDS (Vanderbilt University, Nashville, Tennessee, USA) *Arch Ophthalmol* 1981;**99:**1392-5.

Production of treponemicidal concentration of penicillin in cerebrospinal fluid
EMC DUNLOP, S AL-EGAILY, AND ET HOUANG (Whitechapel Clinic, London Hospital, London, UK) *Br Med J* 1981;**283:**646.

Current features of the clinical picture and course of syphilis
RS BABAYANTS AND BI ZUDIN (Moscow, USSR). *Vestn Dermatol Venerol* 1981; June:4-7.

Syphilitic phlebitis simulating branch vein occlusions
LA LOBES AND JC FOLK (Eye and Ear Hospital, Pittsburgh, Pennsylvania, USA). *Ann Ophthalmol* 1981;**13:**825-8.

Primary syphilis of the tonsil: presentation of four cases
WA VIERS (Washington University School of Medicine, St Louis, USA). *Laryngoscope* 1981;**91:**1507-11.

Syphilis (pathology and experimental)

Plasmid DNA in *Treponema pallidum* (Nichols): potential for antibiotic resistance by syphilis bacteria
MV NORGARD AND JN MILLER (Department of Microbiology and Immunology, University of California, USA). *Science* 1981;**213:**553-5.

Treponemal DNA was obtained from purified *T pallidum* (Nichols) by treatment with lysozyme in a hypotonic lysis buffer followed by ribonuclease. Column chromatography on Sephacryl S-300 to remove impurities showed a peculiar shoulder just before the peak due to RNA. Further analysis showed this to be DNA which was not the same as that found in rabbit mitochrondrial cells. The molecules were examined by transmission electron microscopy and found to be circular, 3·62 μm in length, and 7·5 × 10^6 daltons in weight. The authors think that approximately 10 plasmids existed in each cell of *T pallidum*.

The importance of this finding is in the role that plasmids play in conferring resistance to antibiotics. It could mean that resistance to penicillin in *T pallidum* is now a possibility in the near future.

G D Morrison

Fluorescent treponemal antibody-absorption double-staining procedure
E HUNTER, BJ PENDER, EJ KENNEDY, *ET AL* (Center for Disease Control, Atlanta, Georgia, USA). *J Clin Microbiol* 1981;**14:**184-8.

Gonorrhoea (clinical)

Gonococcal urethritis with bilateral tysonitis and periurethral abscess
S SUBRAMANIAN (General Infirmary, Leeds, UK). *Sex Transm Dis* 1981;**8:**77-8.

Screening for gonorrhoea in a prenatal clinic in South East Asia
RH GOH, YFN GEOW, AND SK TEOH (Department of Obstetrics-Gynaecology, University of Malaya, Kuala Lumpur, Malaysia). *Sex Transm Dis* 1981;**8:**67-9.

Gonorrhoea (microbiology)

Auxotypes and antibiotic susceptibilities of *N gonorrhoeae* from women with acute salpingitis: comparison with gonococci causing uncomplicated genital tract infection

DL DRAPER, JF JAMES, WK HADLEY, AND RL SWEET (University of California, San Francisco, USA). *Sex Transm Dis* 1981; **8**:43-50.

Electron microscopy of the gonococcal capsule

JO HANDLEY, KR POWELL, NL SALOMONSKY, AND RR RODEWALD (University of Virginia, Charlottesville, USA). *J Infect Dis* 1981; **143**:796-802.

Primary isolation of *N gonorrhoeae* on haemoglobin-free NYC medium

PA GRANATO, C SCHNEIBLE SMITH, AND LB WEINER (Syracuse, New York, USA). *J Clin Microbiol* 1981; **14**:206-9.

β-lactam susceptibility of *Neisseria gonorrhoeae* isolated from pelvic inflammatory disease

G PINON, R QUENTIN, P LAUDAT, AND R VARGUES (Bretonneau Hospital, Tours, France). *Antimicrob Agents Chemother* 1981; **20**:260-1.

Comparative in vitro activity of Mk-0366 and other selected oral antimicrobial agents against *Neisseria gonorrhoeae*

MY KHAN, Y SIDDIQUI, AND RP GRUNINGER, (Hennepin County Medical Center, Minneapolis, USA). *Antimicrob Agents Chemother* 1981; **20**:265-6.

Effects of β-lactam antibiotics on peptidoglycan synthesis in growing *Neisseria gonorrhoeae* including changes in the degree of O-acetylation

JK BLUNDELL AND HR PERKINS (Department of Microbiology, University of Liverpool, Liverpool, UK). *J Bacteriol* 1981; **147**: 633-41.

Ampicillin treatment of *N gonorrhoeae* in vivo: an experimental study in rabbits

T ELMROS, SE HOLM, E KJELLBERG, AND B WINBLAD (Umea University, Umea, Sweden). *Acta Pathol Microbiol Scand (B)* 1981; **89**:143-8.

Evaluation of the Phadebact gonococcus test in the identification of *Neisseria gonorrhoeae* in a routine diagnostic laboratory

DS TOMPKINS, BBG NEHAUL, CAF SMITH, AND EM COOKE (Department of Microbiology, University of Leeds, Leeds). *J Clin Pathol* 1981; **34**:1106-9.

The Phadebact gonococcus test, a coagglutination procedure for confirming the identity of presumptive *N gonorrhoeae* isolates, was evaluated under normal working conditions in a routine diagnostic laboratory and compared with an immunofluorescence technique. Of 166 isolates of *N gonorrhoeae* from urogenital, rectal, and pharyngeal sites, 164 gave a positive coagglutination reaction and one of the two negative isolates gave a positive reaction on retesting after subculture. There were no cross-reactions with other organisms tested. This was in contrast to the immunofluorescence technique; with this method, three of 46 isolates of *N meningitidis* fluoresced brightly and a further 17 isolates of *N meningitidis* gave reactions that were difficult to interpret.

The coagglutination test is rapid, simple, and a more specific alternative to immunofluorescence.

Authors' summary

Gonorrhoea (therapy)

Comparative in vitro activity of eight cephalosporins on 108 strains of *N gonorhoeae* and 60 strains of *N meningitidis*

A THABAUT, JL DUPOSOIR, AND P SALLOU (Hôpital Militaire, Begin, St Mandé, France). *Chemotherapy* 1981; **27** suppl 1: 19-24.

Office management of gonococcal urethritis

HH NEUMANN (New Haven Department of Health, New Haven, USA). *Sex Transm Dis* 1981; **8** suppl 2:100-4.

A three-day doxycycline regimen for treatment of gonorrhoea

HH NEUMANN, F KAY, AND C FENGER (Department of Health, New Haven, USA). *Sex Transm Dis* 1981; **8**:73-4.

Comparative study between gentamicin and spectinomycin in the treatment of infections due to penicillin-resistant gonococci

SS PAREEK AND MNH CHOWDHURY (University of Riyadh, Saudi Arabia). *Curr Ther Res* 1981; **30**:177-80.

Non-specific genital infection

Epidemiology and control of non-gonococcal urethritis and genital chlamydial infections—a review

FN JUDSON (Disease Control Service, Denver, Colorado, USA). *Sex Transm Dis* 1981; **8** suppl 2:117-26.

Evaluation of doxycycline hyelate in the treatment of non-gonococcal urethritis

PJ McNEIL, NJ FIUMARA, JJ CALIANDO, *ET AL* (Massachusetts Department of Public Health, Boston, USA). *Sex Transm Dis* 1981; **8** suppl 2:127-31.

Chlamydial infection of the female genital tract with emphasis on pelvic inflammatory disease—a review of Scandinavian studies

PA MÅRDH, BR MØLLER, AND J PAAVONEN (University of Lund, Sweden). *Sex Transm Dis* 1981; **8** suppl 2:140-55.

Chlamydial infections. A worldwide problem: epidemiology and implications for trachoma therapy

J SCHACHTER AND CR DAWSON (George Williams Hooper Foundation, University of California, San Francisco, USA). *Sex Transm Dis* 1981; **8** suppl 2:167.

Recovery and identification of human genital tract mycoplasma

D TAYLOR-ROBINSON AND PM FURR (Clinical Research Centre, Harrow, UK). *Isr J Med Sci* 1981; **17**:648-53.

Therapy for non-gonococcal urethritis: double-blind randomised comparison of two doses and two durations of minocycline

WR BOWIE, R ALEXANDER, JB STIMSON, JF FLOYD, AND KK HOLMES (Department of Medicine, University of British Columbia, Victoria, BC, Canada). *Ann Intern Med* 1981; **95**:306-11.

Chlamydial genital infections—manifestations and management

RE ELLIS (University of Texas, San Antonio, Texas, USA). *South Med J* 1981;**74**:809-13.

Cultural method for large-scale screening for *Chlamydia trachomatis* genital infection

H MALLINSON, S SIKOTRA, AND OP ARYA (Regional Public Health Laboratory, Fazakerley Hospital, Liverpool, UK). *J Clin Pathol* 1981;**34**:712-8.

Established methods for the culture of *C trachomatis* are time-consuming, labour intensive, and not generally suitable for screening purposes. This method incorporates two new ideas. Firstly, a microplate consisting of 96 individual wells in which idoxuridine-treated McCoy cells are grown as monolayers is used as the culture system, and, secondly, periodic acid-Schiff reagent is used for staining (tissue culture cells stain blue and chlamydial inclusions bright magenta). The microplate may be processed as an individual unit (cross-contamination between the closely spaced wells being avoided by careful technique) obviating the need to centrifuge and stain cultures individually. PAS staining enabled direct microscopy to be used and compared favourably with darkfield microscopy of Giemsa-stained conventional coverslip cultures, the inclusions being more readily apparent.

Comparison of inclusion counts on coverslip and microplate wells showed the latter to be about 7·6-fold less sensitive (mainly due to the smaller inoculum used). In practice this decreased sensitivity would only lead to false-negative results on those specimens showing 1-7 inclusions by the coverslip methods. It has been shown that such low counts occur in only a small proportion of chlamydia-positive patients. It is therefore claimed that if the microplate method is applied to women attending STD clinics, for example, it would detect 91·8% of those in whom *C trachomatis* infection could be demonstrated by conventional methods.

This technique could be applied to the examination of 384 (4 × 96 wells) specimens concurrently and is sufficiently sensitive to form the basis of a chlamydial culture service for patients attending STD clinics.

J R Willcox

Reiter's disease

Reiter syndrome in association with enteritis due to *Campylobacter foetus* ssp *jejuni*

A PONKA, J MARTIO, AND TU KOSUNEN (Department of Bacteriology and Immunology, University of Helsinki, Finland). *Ann Rheum Dis* 1981;**40**:414-5.

Reiter's syndrome—a review and case report

MA TOZZI, R STAMM, AJ BIGELLI, AND DJ HART (Cleveland, Ohio, USA). *J Am Podiatry Assoc* 1981;**71**:418-22.

Cell-mediated immune responses of synovial mononuclear cells to sexually transmitted, enteric, and mumps antigens in patients with Reiter's syndrome, RA, and AS

DK FORD, DM Da ROSA, AND P SHAH (Arthritis Center, Vancouver, Canada). *J Rheumatol* 1981;**8**:220-33.

Reiter's syndrome—evaluation of preliminary criteria for definite disease

RF WILLKENS, FC ARNETT, T BITTER, *ET AL* (University of Washington, Seattle, USA). *Arthritis Rheum* 1981;**24**:844-9.

A retrospective evaluation of 83 patients with Reiter's syndrome (RS) and 166 controls with arthritis was carried out to assess the preliminary criteria for definite RS. Data analysis was based on the statement that Reiter's syndrome consists of an episode of peripheral arthritis of more than one month's duration occurring in association with urethritis or cervicitis or both. During the initial episode of RS, 70 of the 83 patients with RS satisfied the criteria, yielding a sensitivity of 84·3%.

Authors' summary

Candidosis

Sensitivity of *Candida* spp to 5-fluorocytosine, amphotericin B, and imidazoles

E SAKELL AND A RIDES (Institute of Microbiology, University of Lausanne, Switzerland). *Schweiz Med Wochenschr* 1981;**111**:1367.

Tissue response to the blastospores and hyphae of *Candida albicans* in mice

ZA EVANS (University of Alabama, Huntsville, Alabama, USA). *J Med Microbiol* 1981;**14**:307-20.

Responses of human peripheral lymphocytes to soluble and insoluble antigens of *Candida albicans*

SMM GETTNER AND DWR McKENZIE (Pahlavi University, Shiraz, Iran). *J Med Microbiol* 1981;**14**:333-40.

Genital herpes

Concurrent oral and genital infection with an identical strain of herpes simplex virus type I restriction endonuclease analysis

JA EMBIL, FR MANUEL, AND ES McFARLANE (Halifax, Nova Scotia, Canada). *Sex Transm Dis* 1981;**8**:70-2.

Inhibition of herpes simplex virus transformed and non-transformed cells by acycloguanosine: mechanisms of uptake and toxicity

RL DAVIDSON, ER KAUFMANN, CS CRUMPACKER, AND LE SCNIPPER (Children's Hospital Medical Center, Boston, USA). *Virology* 1981;**113**:9-19.

Genital herpes simplex

IS TUMMON, DKL DUDLEY, AND JH WALTERS (Ottawa, Canada). *Can Med Assoc J* 1981;**125**:23-9.

Risk of recurrence after first episodes of genital herpes

WC REEVES, L COREY, HG ADAMS, LA VONTVER, AND KK HOLMES (Virology Laboratory, Children's Orthopaedic Hospital, Seattle, USA). *N Engl J Med* 1981;**305**:315-9.

To define risk factors associated with recurrent genital herpes simplex virus infection caused by either type 1 or 2 herpesvirus (HSV-1 or HSV-2), 137 patients with a first symptomatic episode of the disease and 87 with a recurrent episode were studied prospectively. First episodes were divided into 78 primary infections (no antibodies present). HSV-1 infections were less frequent and less likely to recur than HSV-2 infections. Of primary first episodes, 15% were caused by HSV-1 compared with 3%

of non-primary first episodes and 2% of recurrent episodes. Moreover, during follow up of first-episode patients, only 14% of HSV-1 infections recurred compared with 60% of HSV-2 infections. Recurrences were more likely to follow an index recurrent episode than an index first episode, whether primary or non-primary, and to occur in men than in women. Among patients with primary HSV-2 infections the probability of recurrence was directly related to the titre or presence of neutralizing antibody to HSV-2 in convalescent-phase serum.

Authors' summary

Other sexually transmitted diseases

Sexual transmission of enteric protozoa and helminths in a venereal disease clinic population

SC PHILIPS, D MILDVAN, DC WILLIAM, *ET AL* (Beth Israel Medical Center, New York, USA). *N Engl J Med* 1981;**305**:603-6.

The prevalence of enteric protozoan and helminthic infections and the associations between infection and gender, sexual preference, and sexual practices in 180 consecutive patients at a venereal disease clinic were examined. Of 163 men, 29 were infected with one or more enteric parasites. None of the 17 women had an enteric infection. The prevalence of infections with *Entamoeba histolytica* or *Giardia lamblia* (or both) was 21·5% in homosexual men, 6·2% in bisexual men, and 0% in heterosexual men. There were significant associations between oro-anal sexual contact and infection with *E histolytica* ($p<0{\cdot}01$) or with helminths ($p<0{\cdot}05$). Homosexuality and oro-anal contact were the most important risk factors for *E histolytica, G lamblia,* and helminthic infections. Hyperendemic enteric protozoan infection rates in homosexual men appear to be related to three factors: the original endemic level in the general population; the prevalence of sexual acts that facilitate transmission; and the frequency of exposure to an infected person.

Authors' summary

Human papilloma virus type 1 purified from human genital warts

MJ STAQUET, J VIAC, R BUSTAMENTE, AND J THIVOLET (Dermatology Clinic, Hôpital Edouard-Herriot, Lyon, France). *Dermatologica* 1981;**162**:213-9.

Human papilloma virus (HPV) was isolated from a pool of genital warts. The electrophoretic mobility of virion proteins was studied by SDS polyacrylamide gel electrophoresis and showed the same pattern as that obtained with HPV-1. The analysis of DNA after restriction enzyme digestion with the endonucleases Hind III and Hae III and nucleic acid hybridisation did not show any difference with HPV-1. The viral particles were agglutinated by anti-HPV-1 serum, as shown by the electron microscopic particle agglutination test. Furthermore, the immunological properties of this virus were investigated with guinea pig antiserum. Serologically, no antigenic cross-reaction between common and genital wart viruses was shown by immunodiffusion and immunofluorescence tests, whereas cross-reactions were detected between plantar and genital wart viruses. These results possibly indicate that HPV-1 can induce plantar warts as well as genital warts.

Authors' summary

Public health and social aspects

Syphilis and gonorrhoea: epidemiology update

E KAKLAMANI, D TRICHOPOULOS, *ET AL* (University of Athens, Greece). *Paediatrician* 1981;**10**:207-15.

Penicillinase-producing gonococci in Britain

AE JEPHCOTT, N DICKGIESSER AND AN McLEAN (Public Health Laboratory Service, Bristol, UK). *Lancet* 1981;ii:247-8.

The gay report on sexually transmitted diseases

WW DARROW, D BARRETT, K JAY, AND A YOUNG (Center for Disease Control, Atlanta, Georgia, USA). *Am J Public Health* 1981;**71**:1004-11.

Serogrouping and auxotyping for epidemiological study of β-lactamase-producing *Neisseria gonorrhoeae* strains isolated in Sweden

S BYGDEMAN, I KALLINGS, AND D DANIELSSON (Karolinska Institute, Stockholm, Sweden). *Acta Derm Venereol* 1981;**61**:329-34.

Public health implications and control of sexually transmitted chlamydial infections

HH HANDSFIELD, WE STAMM, AND KK HOLMES (United States Public Health Service, Seattle, Washington, USA). *Sex Transm Dis* 1981;**8**:85-6.

Initiation of the sexually transmitted diseases prevention/training program

S MARGOLIS (Center for Disease Control, Atlanta, USA). *Sex Transm Dis* 1981;**8**:87-8.

Failure to identify venereal disease in a lesbian population

P ROBERTSON AND J SCHACHTER (Santa Monica, California, USA). *Sex Transm Dis* 1981;**8**:75-6.

An evening clinic specifically designated for a study into sexually transmitted diseases and the prevalence of cervical dysplasia in sexually active lesbians (SAL) was set up for three months in the autumn of 1978 at San Francisco General Hospital. An SAL was defined as a woman who had been sexually active solely with women in the past six months. Of 148 women, 13% had been sexually active with bisexual women; the remainder were exclusive lesbians. In the previous year the mean number of partners was 2·3 (range 1-30) and 53% had one partner only. Eighty-nine per cent had had previous coital experience with men. Ages ranged from 17-51 years, mean 28 years; 92% were Caucasian.

Recognised routine methods were used to test for syphilis, cervical gonorrhoea, and both herpes simplex and *Chlamydia trachomatis* infections of the cervix. Cervical cytology was studied using the Papanicolau smear. No apparent sexually transmitted diseases were found. Cervical atypia, ranging from mild dysplasia to carcinoma in situ, was detected in four (2·7%) women. The authors suggest that routine screening for venereal disease may not be cost-effective in a lesbian population, but the routine examination of Papanicolau smears should be encouraged.

The methods used for screening for sexually transmitted diseases lack tests for detecting *T vaginalis* and *C albicans* infections as well as urethral infections.

Michael Waugh

Miscellaneous

Therapeutic decisions in the treatment of sexually transmitted diseases: an overview
MF REIN (University of Virginia, Charlottesville, Virginia, USA). *Sex Transm Dis* 1981; **8** suppl 2: 93-9.

Diagnosis and treatment of pelvic inflammatory disease in the emergency room
RL SWEET (San Francisco General Hospital, San Francisco, USA). *Sex Transm Dis* 1981; **8** suppl 2: 156-63.

Pelvic inflammatory disease in the United States: incidence and trends in private practice
RK St JOHN, J BLOUNT, AND O JONES (Center for Disease Control, Atlanta, Georgia, USA). *Sex Transm Dis* 1981; **8**: 56-61.

Pelvic inflammatory disease in the United States: epidemiology and trends among hospitalised women
RK St JOHN, O JONES, JH BLOUNT, AND AA ZAIDI (Center for Disease Control, Atlanta, Georgia, USA). *Sex Transm Dis* 1981; **8**: 62-6.

Granuloma venereum—a rare imported venereal disease
H HOYER AND K WEISMANN (Finsen Institute of Dermatology, Copenhagen, Denmark). *Hautarzt* 1981; **32**: 374-5.

Rapid identification of *Corynebacterium vaginale* in non-purulent vaginitis
JI WELLS AND SH GOEI (King Edward Memorial Hospital for Women, Perth, Australia). *J Clin Pathol* 1981; **34**: 917-20.

To assess the accuracy of a simple set of tests which can be performed on suspected colonies of *Corynebacterium vaginale* on the day of isolation, 1402 unselected women attending gynaecological clinics were studied. Samples from the endocervix and vaginal fornices were cultured on selective media for *Neisseria gonorrhoeae, Trichomonas vaginalis, Candida* and related yeasts, anaerobic pathogens, and *C vaginale* using the modified peptone starch dextrose (PSD) agar of Dunkleberg and growth under anaerobic conditions compared with 5% CO_2. Suspicious colonies (white with slightly darkened centres and displaying starch hydrolysis) were Gram-stained, Albert-stained, and tested for catalase production. Those isolates that were Gram-variable diphtheroid bacilli producing metachromatic granules and were catalase-negative were recorded as *C vaginale*.

Of the 1402 patients, *C vaginale* was identified in 380 but was only considered to be pathogenic in 70 who complained of an abnormal vaginal discharge. Of these 70, *C vaginale* was confirmed in 66 using the described tests, a 94% rate of accurate identification. When the hydrolysis of hippurate, specific for *C vaginale*, was added the accuracy was increased to more than 97%. All the isolates grew under anaerobic conditions but only 70% under CO_2, and a heavy virtually pure growth of *C vaginale* was found in 66 of the 70. The appearance of clue cells in the vaginal discharge correlated poorly with the culture results (present in about 20%), but a typical Gram-stained appearance of (a) few or no leucocytes, (b) many epithelial cells, and (c) masses of Gram-variable coccobacilli was found in all 70.

With the increasing acceptance of the role of *C vaginale* in non-purulent vaginitis the advantages of rapid identification are obvious; however, its role in such a subjective complaint (82% were allegedly asymptomatic in this survey) requires further elucidation.

R S Pattman

M.S.S.V.D.

The Medical Society for the Study of Venereal Diseases

11 Chandos Street, Cavendish Square, London W1M 0EB

President: Dr. Jack Barrow

The object of the Society is to bring together those interested or engaged in the diagnosis, treatment, and public health aspects of venereal diseases. The Society meets regularly in London for the reading of papers, discussions, and presentation of cases. An out-of-London meeting is held in the Spring.

Annual subscription for membership of the Society is £20 for all members.

Applications for membership are very welcome and forms can be obtained from the Honorary Secretary, Dr. M. A. Waugh, Department of Genito-Urinary Medicine, The General Infirmary Great George Street, Leeds LS1 3EX.

Proposed Programme 1982

Friday 30th April 1982	Candidiasis — Immunology Prof. J. R. Hobbs — Mycosis with Special Reference to Genital Candidiasis Prof. D. W. Mackenzie
Friday 21st-23rd May 1982	Spring Meeting in Jersey

The 31st General Assembly of the International Union against the Venereal Diseases and the Treponematoses will convene in Dublin on June 25-29, 1982.

Applications for papers should be received by:

Dr J. R. W. Harris
The Praed Street Clinic
St Mary's Hospital
Praed Street, London W2 1NY

not later than May 31st, 1982

Sexually Transmitted Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $25.00 outside the United States ($22.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $33.00 and $36.00 in the United States and $37.00 and $40.00 in all other countries.

William O. Harrison, M.D.
American Venereal Disease Association
P.O. Box 22349
San Diego, California 92122 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $25.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases.*

☐ I wish to enter a Non-Member or institutional subscription ($33.00 and $36.00 U.S., all other countries $37.00 and $40.00).
(Mail non-member or institutional subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105).

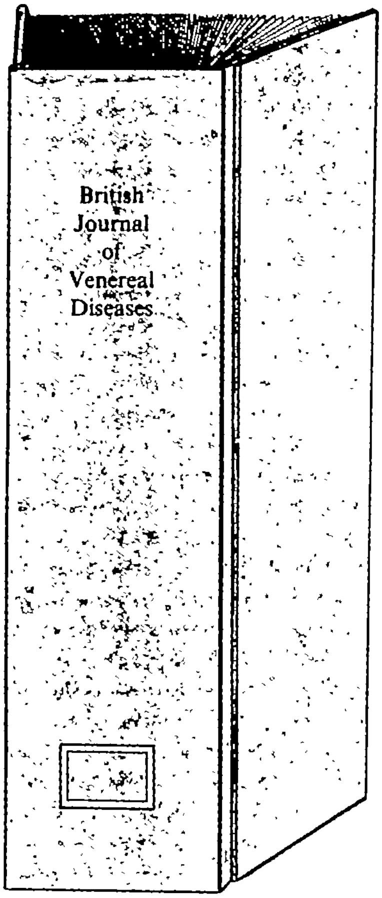
Name ____________________

____

Address ____________________

City __________ State Country __________ Zip __________

Available January 1982



'CORDEX'

SELF-BINDING CASES

for easy and instant reference

Made to hold two volumes (12 issues) of your **BRITISH JOURNAL OF VENEREAL DISEASES.** Each case is handsomely bound with a colour to complement the journal. Spring nylon cords, to carry the journals, slide freely along the spine of the binder. The name of the journal is gold-blocked on the spine. Date labels are also provided.

Price: Inland £4.00; Overseas US$12.00
including postage (air mail overseas) and packing

ORDER NOW

From: The Subscription Manager,
BRITISH JOURNAL OF VENEREAL DISEASES
BMA House, Tavistock Square, London WC1H 9JR

British Journal of Venereal Diseases April 1982 Vol 58 No 2

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Editorial: statistical significances—use and misuse
A McMILLAN *page* 71

IV Harrison Lecture 1981: the international venereological scene as viewed by Harrison and St Mary's
R R WILLCOX *page* 72

Higher medical training in venereology
C B S SCHOFIELD *page* 86

Role played by Italy in the history of syphilis
M A WAUGH *page* 92

Inhibition of *Neisseria gonorrhoeae* isolates by Martin-Lewis medium: epidemiology, susceptibility profile, and plasmid analysis
M A MELLER, P ANDERSON, J W PARKER, AND H H ROHRER *page* 96

Treatment of pharyngeal gonorrhoea due to β-lactamase-producing gonococci
MAGNUS LINDBERG, OLOF RINGERTZ, AND ERIC SANDSTRÖM *page* 101

Effects of tampon components on growth and dissemination of *Neisseria gonorrhoeae*
R J ARKO, K H WONG, K G FINLEY-PRICE AND J K RASHEED *page* 105

Rapidity and reliability of gonococcal identification by coagglutination after culture on modified New York City medium
H YOUNG AND A McMILLAN *page* 109

Epidemiological treatment of gonorrhoea and non-specific genital infection in female sexual contacts: current practices in STD clinics in England and Wales
ELIZABETH M BELSEY *page* 113

Rosaramicin and tetracycline in the treatment of non-gonococcal urethritis: a comparison of clinical and microbiological results
J F DARNE, G L RIDGWAY, AND J D ORIEL *page* 117

Metronidazole in a single dose for the treatment of trichomoniasis: failure of a 1-g single dose
THOMAS W AUSTIN, ELIZABETH A SMITH, RIAD DARWISH, EDWARD D RALPH, AND FREDERICK L M PATTISON *page* 121

Treatment of vaginal candidosis with a single 500-mg clotrimazole pessary
IAN MILSOM AND LARS FORSSMAN *page* 124

Balanoposthitis associated with *Gardnerella vaginalis* infection in men
G R KINGHORN, B M JONES, F H CHOWDHURY, AND I GEARY *page* 127

Correspondence *page* 130

Book review *page* 133

Abstracts *page* 134

ASTM CODEN: BJVDAK 58 (2) 71-138 (1982)

ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

NCC
16.7.82

June 1982 Vol. 58 No. 3

British Journal of
Venereal Diseases

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

A E WILKINSON
(Editor)

B A EVANS
(Assistant Editor)

A McMILLAN
(Assistant Editor)

G D MORRISON
(Assistant Editor, Abstracts)

J BARROW
(President, MSSVD)

M WAUGH
(Hon Sec, MSSVD)

M W ADLER

G CAUSSE

G CSONKA

L M DRUSIN

J M KNOX

I PHILLIPS

E STOLZ

R N THIN

J WALLIN

EDITOR,
British Medical Journal

P D SIMMONS
(Indexer)

SHUNA JUDGE
(Technical Editor)

This *Journal,* founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A McMillan, Dept of Genitourinary Medicine, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals,* available from the Publishing Manager, *British Medical Journal,* BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; 55: 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS, Rodin P. *Venereal Diseases.* 4th ed. London: Baillière and Tindall, 1980.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 214-22.

PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £31.00 in the United Kingdom and Republic of Ireland and US$76.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US$76.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal,* 1172 Commonwealth Avenue, Boston, Mass. 02134. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

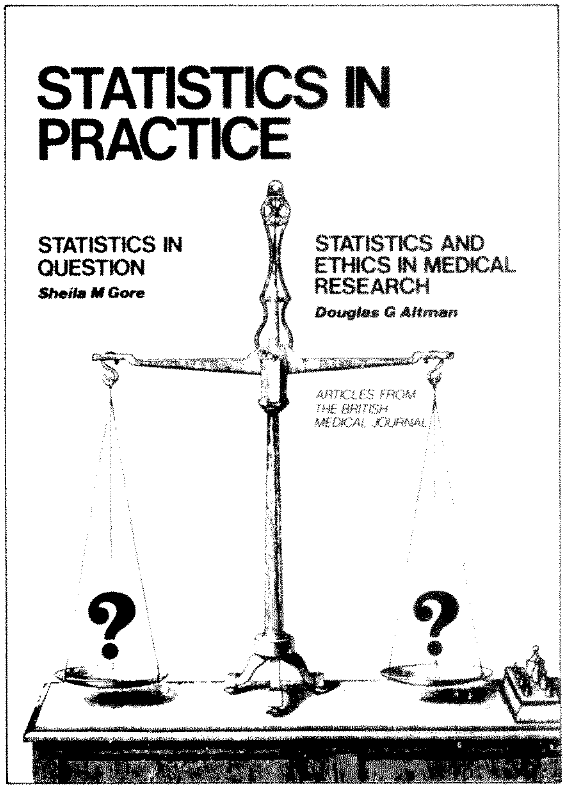
NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases,* BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1982 by the *British Journal of Venereal Diseases.* This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.

Just published

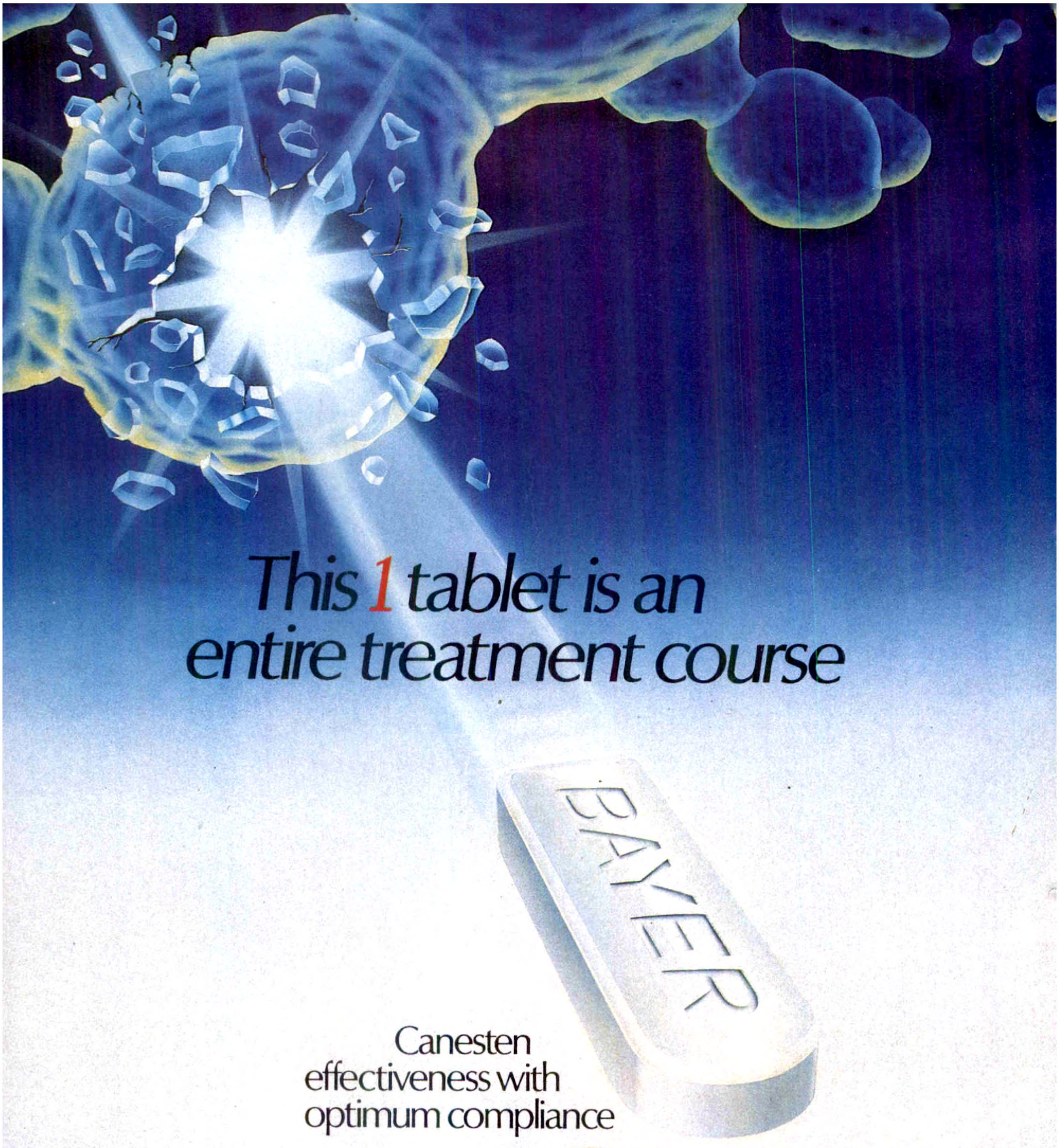
STATISTICS IN PRACTICE

No doctor can afford to ignore statistics: most modern medical research uses statistics. This important and authoritative book, which is a collection of articles that have appeared in the BMJ, provides clear information on designing studies, applying statistical techniques, and interpreting studies that use statistics. It can be easily understood by those with no statistical training and should be read by all those who want to keep abreast of new developments.


STATISTICS IN PRACTICE
STATISTICS IN QUESTION
Sheila M Gore
STATISTICS AND ETHICS IN MEDICAL RESEARCH
Douglas G Altman
ARTICLES FROM THE BRITISH MEDICAL JOURNAL
?
?

Price: Inland £7.00
Overseas US$27.50*
(Inland £6.00;
Overseas US$25.00*
for BMA members)
**including air mail postage*

Order your copy now
From: The Publisher
British Medical Journal
BMA House
Tavistock Square
London WC1H 9JR
or any leading bookseller


This 1 tablet is an entire treatment course
BAYER
Canesten effectiveness with optimum compliance

Canesten® 1

clotrimazole

Singularly effective in vaginal candidiasis

Prescribing Information: Presentations. *Vaginal Tablets* Canesten is available either as 200mg vaginal tablets in packs of three with applicator or as 100mg vaginal tablets in packs of six with applicator, or as a single 500mg vaginal tablet with applicator (Canesten 1). *Vaginal Cream* 2.0% clotrimazole cream in tubes of 35g with six applicators. *Canesten Duopak* Six 100mg vaginal tablets with applicator plus 20g cream containing 1.0% clotrimazole. **Dosage.** *Vaginal Tablets 100mg* Two vaginal tablets should be inserted at night for three consecutive days. Alternatively, one vaginal tablet daily for six days. *Vaginal Tablets 200mg* One vaginal tablet should be inserted at night for three consecutive days. *Vaginal Tablets 500mg (Canesten 1)* A single vaginal tablet to be inserted preferably at night. *Vaginal Cream* One filled applicator (5g) should be inserted twice daily for three consecutive days or once daily for six consecutive days preferably at night. *Duopak* Vaginal Tablets 100mg as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to prevent re-infection. **Side effects.** Rarely patients may experience transient local mild burning or irritation. **Basic NHS Costs.** 6 x 100mg vaginal tablets with applicator £2.13; 3 x 200mg vaginal tablets with applicator £1.99; 1 x 500mg vaginal tablet with applicator £2.50: Cream 35g with applicators £4.16; Duopak £3.47. **Product Licence Numbers.** *Vaginal Tablets 100mg,* PL0010/0015, *Vaginal Tablets 200mg,* PL0010/0072, *Vaginal Tablets 500mg,* PL0010/0083, *Vaginal Cream,* PL0010/0077. *Duopak,* PL0010/0016 and PL0010/0015.

Further information is available from:
Bayer UK Limited
Pharmaceutical Division
Haywards Heath
West Sussex RH16 1TP
Telephone: Haywards
Heath (0444) 414161

*Registered trade mark of Bayer, Germany.

Br J Vener Dis 1982; **58**: 139-42

Effects of anaerobic and microaerophilic conditions of extraction and incubation on the survival of Treponema pallidum in vitro

G H W WONG, B M STEINER, AND S R GRAVES

From the Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran, Victoria, Australia

SUMMARY *Treponema pallidum* extracted from infected rabbit testes under anaerobic conditions survived longer in vitro than those extracted under aerobic conditions. Anaerobically extracted treponemes were incubated anaerobically for 0, 12, 24, 36, or 48 hours and then exposed to microaerophilic conditions (3% oxygen) for further incubation. Treponemes transferred to microaerophilic conditions after 36 or 48 hours' anaerobic incubation maintained significantly greater viability compared with those kept under constant microaerophilic conditions, although there was no difference after 12 or 24 hours. *T pallidum* incubated under constant anaerobic conditions, however, usually maintained greater viability than those kept under constant microaerophilic conditions.

These results suggest that *T pallidum* is sensitive to oxygen toxicity both during initial extraction from orchitic rabbit testes and subsequent incubation in vitro. In the latter case, it can be partially protected by a period of anaerobic incubation in vitro, before exposure to microaerophilic conditions.

Introduction

Treponema pallidum has little ability to handle oxygen toxicity in vitro. Although high concentrations of oxygen enhanced the metabolism of *T pallidum* in vitro,[3 4] prolonged survival and growth have been observed only under low oxygen concentrations[5 6] and electronegative redox potentials.[1]

The survival of *T pallidum* in vitro has been prolonged by several methods—presumably by reducing the toxicity of oxygen—such as the addition of reducing agents,[7-9] co-incubation with tissue culture cells,[9] and the addition of oxygen-protective enzymes (unpublished data).

In this paper we compared the survival of *T pallidum* in vitro after either anaerobic or aerobic extraction from infected rabbit testes on the assumption that oxygen toxicity was minimal in the former case. We also tested the effect of a variable period of incubation under anaerobic conditions before the treponemes were exposed to 3% oxygen to determine whether they were more sensitive to oxygen toxicity immediately after extraction from infected rabbit testes or at sometime later.

Materials and methods

The Nichols strain of *T pallidum* was maintained by intratesticular passage in rabbits. *T pallidum* was harvested within two days of orchitis appearing in the rabbit, which was killed by an intravenous injection of 3 ml sodium pentobarbitone (200 mg/ml). The testes were removed aseptically and cut into longitudinal and transverse slices with sterile scissors.

AEROBIC EXTRACTION

After initial slicing, the testes were further cut and minced in a sterile Petri dish under aerobic conditions in a laminar flow cabinet. The first extract, containing large amounts of tissue debris and erythrocytes, was discarded. The minced testes were then eluted with reduced medium (30 ml) and the extraction completed within 30-60 minutes. When the yield of treponemes was very low or the testicular tissue haemorrhagic or both, the treponemes were not

Address for reprints: Dr G H W Wong, Department of Microbiology, Monash University Medical School, Alfred Hospital, Prahran, Victoria, Australia 3181

Accepted for publication 18 November 1981

used, since they were often found to be of low viability.

ANAEROBIC EXTRACTION

The slices of testicular tissue were placed in a bottle containing reduced medium (30-50 ml) and then flushed with sterile oxygen-free nitrogen. The treponemes were eluted by gentle agitation for 30-60 minutes. The extract was then separated from the testicular tissue and counts made in a bacterial counting chamber by darkfield microscopy. The treponemes obtained by the two extraction methods were adjusted to the same concentration before inoculation into media.

A previously published medium,[1] with 2 mmol/l dithiothreitol instead of sodium thioglycollate was used. The final pH was 7·3 ±0·1, the redox potential −350±50 mV (Ecal), and the incubation temperature 34°C. Anaerobic and microaerophilic incubation have been described.[1]

Percentage motility based on counts of 50-100 treponemes by darkfield microscopy and virulence in rabbits, as shown by the latent period before lesions appeared after intradermal inoculation,[1] were used for determining the viability of *T pallidum* in vitro. Student's *t* test was used to compare the differences between the data.

Results

COMPARISON OF EXTRACTION METHODS ON RETENTION OF VIABILITY AFTER MICROAEROPHILIC INCUBATION

No difference was detected between anaerobically and aerobically extracted treponemes after 24 and 48 hours' incubation. Both the percentage motilities of the cultures and their latent periods for lesion development in rabbits were similar (data not shown). However, significant differences were observed after 72 and 96 hours' incubation in vitro (table I). The anaerobically extracted treponemes maintained significantly higher percentage motility and virulence (shorter latent periods) than aerobically extracted treponemes.

SURVIVAL OF ANAEROBICALLY EXTRACTED TREPONEMES AFTER MICROAEROPHILIC INCUBATION

After anaerobic extraction treponemes were maintained under anaerobiosis for different periods (0, 12, 24, 36, and 48 hours) before incubation in 3% oxygen in nitrogen. The controls were treponemes incubated either anaerobically or under 3% oxygen. After 12 or 24 hours' anaerobic incubation, followed by microaerophilic incubation, there was no significant difference in treponemal survival compared with either control group. However, treponemes held for 36 or 48 hours anaerobically and then exposed to 3% oxygen survived significantly longer, as measured by percentage motility (table II), than those exposed to constant 3% oxygen when sampled at 60, 72, 96, and 120 hours after harvesting. The latent periods of lesion formation in rabbits from treponemes incubated anaerobically for 36 or 48 hours were also significantly shorter than those from treponemes incubated under constant 3% oxygen (table III). Treponemal survival was not significantly enhanced compared with those in the constant anaerobic control group. Treponemes usually maintained viability longer under constant anaerobic conditions than under constant microaerophilic (3% O_2) conditions during prolonged incubation in vitro (tables II and III).

Discussion

The anaerobically extracted treponemes survived longer in vitro than those extracted aerobically, suggesting that *T pallidum* is sensitive to oxygen during the extraction process. Oxygen-derived species (superoxide free radical, hydroxyl free radical, singlet oxygen, and hydrogen peroxide) may act directly or indirectly on: (a) the DNA synthetic complex,[10] the treponemes possibly having lost the ability to initiate or continue DNA synthesis in vitro; (b) the extracellular layer,[11] resulting in loss of virulence in vitro; or (c) the membranes and enzymes,[12] causing a loss of biochemical function in vitro.

TABLE I *Comparison of the effect of anaerobic and aerobic extraction of T pallidum from infected tissue on the survival of motile and virulent treponemes after incubation in vitro*

	% Motility (±SD) after extraction:*			*Latent period† (days±SD) after extraction:*			
Incubation period (hours)	*Anaerobic*	*Aerobic*	*P value*	*Experiment*	*Anaerobic*	*Aerobic*	*P value*
72	55·2±7·6	28·0±13·6	<0·01	1	8·5±1·1	12·1±1·0	<0·001
				2	14·5±0·8	17·3±1·0	<0·001
96	24·3±11·9	6·0±2·8	<0·05	1	15·7±0·8	20·0±0	<0·001
				2	16·6±0·5	18·0±0·8	<0·02

*Mean of counts of 200-600 treponemes in 4-6 samples (50-100 treponemes/sample) from two independent experiments.
†Time between rabbit inoculation and appearance of lesions (mean of 10 lesions for each experiment).
P=probability; P<0·05 is significant (Student's *t* test).

TABLE II *Effect of 36 or 48 hours' anaerobic incubation followed by incubation under 3% oxygen on the viability of T pallidum as measured by the retention of motility*

	% Motility† (±SD) after different incubation periods in vitro (h):							
	Experiment 1			*Experiment 2*			*Experiment 3*	
Incubation conditions	*72*	*96*	*120*	*72*	*96*	*120*	*60*	*72*
Constant 3% O_2 (control)	56±4	29±5	20±1	50±4	35±2	20±3	11±1	6±4
Anaerobic (36 h)*	74±1 P<0·001	57±4 P<0·001	52±1 P<0·001	79±1 P<0·001	66±4 P<0·001	54±2 P<0·001	56±0 P<0·001	45±0·3 P<0·005
Anaerobic (48 h)*	66±4 P<0·01	63±4 P<0·001	57±3 P<0·001	74±1 P<0·001	62±3 P<0·001	47±4 P<0·001	45±2 P<0·005	36±6 P<0·05
Constant anaerobic (control)	64±4 P<0·05	34±5 NS	28±3 P<0·005	70±1 P<0·001	63±3 P<0·001	44±5 P<0·001	43±4 P<0·01	29±2 P<0·02

* *T pallidum* incubated anaerobically and then exposed to 3% O_2 at 34°C in vitro.
†Mean of counts of 100-300 treponemes in 2 or 3 samples (50-100 treponemes/sample) from duplicate or triplicate culture tubes.
P = probability; P<0·05 is significant (Student's *t* test). NS = not significant.

During aerobic extraction, the minced rabbit testes containing the treponemes were directly exposed to atmospheric oxygen. *T pallidum* appears to lack the means of detoxifying oxygen-derived species (unpublished data), thus possibly explaining the more rapid decrease in motility and virulence in vitro compared with anaerobically extracted treponemes (table I). The longer survival of the anaerobically extracted treponemes may have been due to: (a) the prevention of the direct reaction of oxygen with key cellular components of *T pallidum;* (b) a slowing of the rate of production of oxygen-derived free radicals from the medium components; or (c) prevention of a possible oxygen-mediated autolysis of host tissue cells.

Anaerobically harvested treponemes incubated anaerobically for 36 and 48 hours before being exposed to 3% oxygen survived longer than treponemes incubated under constant 3% oxygen (tables II and III) but not longer than the control group under constant anaerobic incubation. This may suggest that *T pallidum* is more sensitive to oxygen during early incubation in vitro.

It is assumed that treponemes which are freshly isolated from the rabbit host are metabolically highly competent. Their endogenous metabolism is presumably very high. A flavoprotein-based energy metabolism, which they are presumed to have,[13] would result in the production of large amounts of hydrogen peroxide.[14] This may explain their high sensitivity to oxygen shortly after extraction from tissue even in the presence of reducing agents. What the oxygen requirements are inside the host and how *T pallidum* avoids the toxicity of oxygen-generated reduction products are as yet unknown. In a recent report of growth of the organism in tissue culture[6] the oxygen concentration was 1·5%. Oxygen may play a very important role in the survival and growth

TABLE III *Effect of 36 or 48 hours' anaerobic incubation followed by incubation under 3% oxygen on the viability of T pallidum as measured by the retention of virulence (latent period of infection)*

	Latent period† (days±SD) of infection after inoculation with treponemes incubated for (h):							
	Experiment 1			*Experiment 2*			*Experiment 3*	
Incubation conditions	*72*	*96*	*120*	*72*	*96*	*120*	*60*	*72*
Constant 3% O_2 (control)	7·3±0·5	11·4±0·5	13·5±0·7	10·4±1·4	14·6±1·0	17·0±0	12·6±1·1	11·2±1·4
Anaerobic (36 h)*	5·8±0·6 P<0·001	9·2±0·4 P<0·001	11·4±0·7 P<0·001	8·4±1·1 P<0·001	12·6±1·0 P<0·001	15·2±0·6 P<0·001	10·7±0·9 P<0·005	9·6±0·5 P<0·005
Anaerobic (48 h)*	6·3±0·7 P<0·01	9·6±0·5 P<0·001	11·6±0·7 P<0·001	9·1±1·4 P<0·05	12·6±0·9 P<0·001	15·5±0·5 P<0·001	10·5±1·8 P<0·01	9·8±0·8 P<0·025
Constant anaerobic (control)	6·8±1·6 NS	11·1±0·7 NS	13·0±0·7 NS	9·2±1·6 NS	12·8±1·1 P<0·001	15·8±0·6 P<0·001	11·0±1·5 P<0·005	9·4±0·5 P<0·005

* *T pallidum* incubated anaerobically and then exposed to 3% O_2 at 34°C in vitro.
†Time between inoculation of rabbits and appearance of lesions (mean of 10-12 lesions).
P = probability; P<0·05 is significant (Student's *t* test). NS = not significant.

of *T pallidum* in vitro,[3 4] but the sensitivity of *T pallidum* to oxygen during extraction from tissue and early incubation in vitro is also very important. Methods used to reduce oxygen toxicity during incubation in vitro may further improve the survival or growth, or both, of *T pallidum* in vitro.

We gratefully acknowledge the assistance provided by Mrs L Drummond and Dr P Sutrave, We would like to thank Professor S Faine and Dr Ben Adler for constructive critical evaluation.

This work was supported by grants from the Australian National Health and Medical Research Council (NH & MRC), the Utah Foundation, the Heiser Program, and Monash University.

References

1. Steiner B, McLean I, Graves S. Redox potential and survival of virulent *Treponema pallidum* under microaerophilic conditions. *Br J Vener Dis* 1981;**57**:295-301.
3. Barbieri J, Cox CD. Pyruvate oxidation by *Treponema pallidum*. *Infect Immun* 1979;**25**:157-63.
4. Lysko PG, Cox CD. Respiration and oxidative phosphorylation in *Treponema pallidum*. *Infect Immun* 1978;**21**:462-73.
5. Graves S, Billington T. An optimum concentration of dissolved oxygen for the survival of virulent *Treponema pallidum* (Nichols strain) under conditions of low oxidation-reduction potential. *Br J Vener Dis* 1979;**55**:387-93.
6. Fieldsteel AH, Cox DL, Moeckli RA. Cultivation of virulent *Treponema pallidum* in tissue culture. *Infect Immun* 1981;**32**:908-15.
7. Norris S, Miller J, Sykes J, Fitzgerald TJ. Influence of oxygen tension, sulfhydryl compounds and serum on the motility and virulence of *Treponema pallidum* (Nichols strain) in a cell-free system. *Infect Immun* 1978;**22**:689-97.
8. Fitzgerald TJ, Johnson RC, Wolfe ET. Sulfhydryl oxidation using procedures and experimental conditions commonly used for *Treponema pallidum*. *Br J Vener Dis* 1980;**56**:129-36.
9. Fitzgerald TJ, Johnson RC, Miller J, Sykes JA. Interaction of *Treponema pallidum* (Nichols strain) with cultured mammalian cells: effects of oxygen, reducing agents, serum supplements and different cell lines. *Infect Immun* 1977;**15**:444-52.
10. Israeli E. Kohn A, Gitelman J. The molecular nature of damage by oxygen to freeze-dried *Escherichia coli*. *Cryobiology* 1975;**12**:15-25.
11. Fitzgerald TJ, Johnson RC. Surface mucopolysaccharides of *Treponema pallidum*. *Infect Immun* 1979;**24**:244-51.
12. Hangaard N. Cellular mechanisms of oxygen toxicity. *Physiol Rev* 1968;**48**:311-73.
13. Lysko P, Cox CD. Terminal electron transport in *Treponema pallidum*. *Infect Immun* 1977;**16**:885-90.
14. Strittmatter CF. Flavin-linked oxidative enzymes of *Lactobacillus casei*. *J Biol Chem* 1959;**234**:2784-800.

Br J Vener Dis 1982;**58**:143-6

Interferon in rabbit sera after inoculation with Treponema pallidum suspensions contaminated with PED virus

K L FENNESTAD,* L BRUUN,* AND S HAAHR†

*From the *Animal Department, Statens Seruminstitut, Copenhagen; and the †Institute of Medical Microbiology, Aarhus University, Aarhus, Denmark*

SUMMARY Paired rabbit sera were examined for the presence of interferon and pathogenicity for rabbits. The sera were obtained before and 48 hours after inoculation with *Treponema pallidum* suspensions of rabbit origin in 12 selected laboratories.

Classical interferon, detectable in dilutions from 1/9 to 1/81, were found in 27 out of 39 postinoculation sera from which the pleural effusion disease (PED) virus was isolated. Serum interferon was not detectable in dilutions >1/9 in 16 virus-negative postinoculation sera or in any of the 55 preinoculation sera. Interferon was found more often in sera from which highly virulent strains of PED virus were isolated than in sera from which strains of low virulence were isolated.

The serum interferon assay provides useful presumptive evidence of contamination of rabbit-passaged treponemes with PED virus, but the assay is least useful when PED virus is present subclinically.

Introduction

Pleural effusion disease (PED) agent or virus occurs as a passenger in *Treponema pallidum* suspensions of rabbit origin, causing intercurrent infection of rabbits, which may vary from a subclinical to a fatal disease.[1-3] The virus is poorly characterised and has not been demonstrated convincingly by culture, electron microscopy, or a specific serological technique[4-6]; it has, however, been found to produce circulating interferon.[5]

During a recent survey of laboratories paired sera were obtained before and 48 hours after intratesticular inoculation with *T pallidum*. These sera were used in a rabbit test for PED virus. By this test the agent was detected in all postinoculation sera from seven laboratories but not in those from five laboratories or in preinoculation sera from all 12 laboratories.[3]

This study reports the results of examining these paired sera for interferon. Furthermore, since the above rabbit test included inoculation of each serum into a rabbit, it was possible to compare the interferon titres of the paired sera with the rabbit pathogenicity of the same sera.

Materials and methods

The paired rabbit sera were received in 1978-79 from selected laboratories (Nos 1-12) and had been stored since then at −70°C. The criteria for selection and geographical location of the laboratories, together with details of the sera and the treponemal inocula, have been described.[3]

Four pairs of sera from the material were excluded from the present study: two pairs (laboratory No 11), because the samples were collected 96 hours after inoculation, one pair (laboratory No 2), because the preinoculation sample was lost, and one pair (laboratory No 3), because the preinoculation sample was inadvertently contaminated. Included in the study were an additional five pairs of sera received from laboratory No 5 after it had been informed of the results of the first set of sera. These last sera came from rabbits inoculated with a treponemal suspension from a rabbit testis which had been stored in liquid nitrogen for five years. The inoculum represented the routinely used substrain of Nichols pathogenic *T pallidum* from an earlier period when laboratory No 5 had a very low intercurrent mortality among inoculated rabbits (personal communication).[7] The final material therefore comprised 55 paired sera.

Address for reprints: Dr K L Fennestad, Animal Department, Statens Seruminstitut, Amager Boulevard 80, DK 2300 Copenhagen S, Denmark

Accepted for publication 23 December 1981

SERUM INTERFERON ASSAY

The sera and tissue culture media were dialysed against Sørensen's buffer pH 2 and after 48 hours at 4°C dialysed back to pH 7·4. Some of the sera were centrifuged at 110 000 × *g* for three hours at 4°C.

The culture used was a continuous rabbit kidney cell line (RK13), originally received from Dr Annelise Godtfredsen, Statens Seruminstitut, Copenhagen. The cells were grown in 1-litre Roux flasks, using Eagle's MEM with 15% calf serum as growth medium and Eagle's MEM with 2% calf serum as maintenance medium. Both media contained 200 IU penicillin, 0·2 mg streptomycin, and 20 IU mycostatin per ml.

A micromethod was used in which Microtest II (Falcon) wells were seeded with RK13-cells, 30 000 cells/well in a volume of 0·1 ml. After growth to confluence, serial three-fold dilutions of the sera or tissue culture media to be tested were made directly in the wells. Twenty-four hours later the cells were challenged with vesicular stomatitis virus (500 plaque-forming units in 0·1 ml/well) and the cytopathic effect was read microscopically after incubation for 24 hours. The interferon titres represented the dilution that reduced the cytopathic effect by 50%. All samples were tested in duplicate rows. An interferon standard of known titre was included on each plate to correct for fluctuations in the sensitivity of the system.

Control interferon was induced in rabbits weighing 2-3 kg by double-stranded polynucleotide (Poly I/Poly C, Miles Laboratories). The compound was dissolved in PBS (pH 7·3) at a concentration of 1000 U/ml. Each rabbit was given 1 ml intravenously. Blood was taken from an ear vein after two hours. The serum was dialysed against Sørensen's buffer pH 2, and after 48 hours at 4°C it was dialysed back to pH 7·4.

The viral inhibitor found in the dilutions of sera was stable at pH 2 for 48 hours, partly inactivated at 56°C for 30 minutes, and inactivated by trypsin. No changes in the viral inhibitory effect was found after centrifugation at 110 000 × *g* for three hours at 4°C. This indicated type 1 interferon or classical interferon.[9]

PATHOGENICITY TEST

To determine the pathogenicity of each serum for rabbits 0·2 ml serum mixed with 0·8 ml of PBS (pH 7·0) was given subcutaneously to one rabbit. All rabbits were albino males, weighing 2-3 kg from the same closed colony (Ssc:CPH). After inoculation the rabbits were observed for clinical signs of disease for a period of 10 days, particularly those characteristic of PED.[10] The clinical response of the individual rabbits was classified according to the severity of the disease: A—fatal disease with necropsy findings characteristic of PED; B—febrile disease with iridocyclitis; C—transient fever within three days after inoculation; and D—no clinical signs of disease. A rectal temperature of 40°C or higher was considered as fever, except in one case of 39·9°C.

VIRUS ISOLATION

The results of isolation of PED virus from the post-inoculation sera have been reported previously[3] but are included in this study.

Results

Tables I and II summarise the results of examining sera obtained before and 48 hours after intratesticular inoculation of rabbits with *T pallidum* suspensions in the 12 laboratories.

PREINOCULATION SERA

Neither PED virus nor interferon was detectable in the individual sera before inoculation with treponemes (table I). When the same sera were examined for rabbit pathogenicity, four sera (from laboratory Nos 2, 5, and 6) produced transient fever (C response), while the remaining 51 sera failed to induce clinical signs of disease (D response).

POSTINOCULATION SERA

In the virus-positive group of laboratories the interferon titres varied from <3 to 81 (table II). An interferon titre of ≥3 was found in 28 out of 39 virus-positive sera. Nevertheless, 11 of the 39 virus-positive sera had titres of <3. In the virus-negative group of laboratories two of the 16 sera showed a titre of 3, while the rest had a titre of <3 (table II). If an interferon titre of ≥9 is considered specific, that is, caused by PED virus infection, 27 out of the 39 virus-positive sera showed the presence of interferon. At least one serum from each virus-positive laboratory showed a titre of ≥9.

TABLE I *PED virus isolation, interferon titres, and rabbit pathogenicity in 55 preinoculation sera*

			Individual sera	
Laboratory No	*No of sera*	*PED virus isolated*	*Interferon titre*	*Pathogenicity**
1-12	55	0	All <3	4C, 51D

* See text

TABLE II *PED virus isolation, interferon titres, and rabbit pathogenicity in 55 postinoculation sera*

Laboratory No	*No of sera*	*PED virus isolated*	*Individual sera* *Interferon titre/pathogenicity**
(Virus-positive)			
2	5	5	9/C, 9/C, 81/B, 3/B, 27/B
3	3	3	<3/A, 27/C, 81/B
5a†	5	5	27/A, 9/A, <3/B, 9/A, <3/C
5b	5	5	<3/C, <3/C, <3/C, 9/D, 9/C
6	8	8	27/C, 27/B, 27/A, 81/A, 27/B, 27/A. 27/B, 27/B
7	4	4	81/C, 27/B, 27/B, 27/B
8	5	5	81/B, <3/B, 27/C, 9/B, <3/B
12	4	4	<3/D, <3/D, 9/C, <3/C
(Virus-negative)			
1	4	0	3/D, <3/D, 3/D, <3/D
4	4	0	<3/D, <3/D, <3/D, <3/D
9	2	0	<3/D, <3/D
10	4	0	<3/D, <3/D, <3/D, <3/D
11	2	0	<3/D, <3/C

* See text
† Laboratory No 5 represented by two sets of sera

The pathogenicity of the postinoculation sera varied in response from A to D in the virus-positive laboratories, whereas a C or D response was obtained with sera from the virus-negative laboratories (table II).

In the figure the interferon titres of the 55 postinoculation sera are grouped according to the clinical response after inoculation of rabbits with the sera. The range and mean of interferon titres of the virus-positive sera were the same for the A and B responses, whereas the titres of the virus-positive sera tended to be lower for the C and D responses. If the A and B responses are grouped together and compared with the group of C and D responses there is a significant difference in titres between the two groups ($P<0·05$, Wilcoxon's two-sample test). This shows a correlation between interferon production and the severity of the disease.

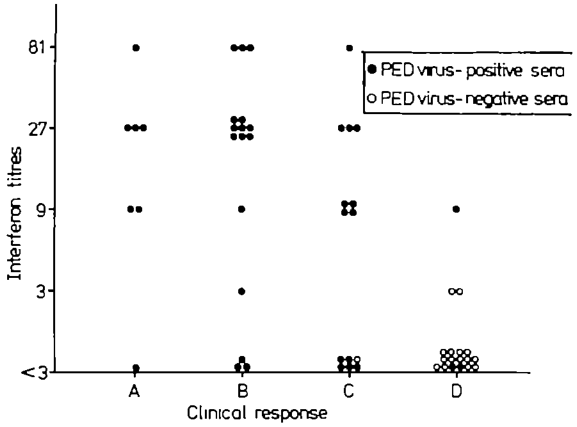


FIGURE *Correlation between interferon titres of postinoculation sera and severity of clinical response after rabbit inoculation.*

Discussion

The idea that the presence of interferon in serum may be used as presumptive evidence of acute viral infections was proposed many years ago[11] and has since been supported by other observations.[12 13] Recently, Matthews and Lawrence[14] found that single "acute" sera gave positive results in 17 out of 45 patients with various confirmed viral infections. They also suggested that the interferon serum assay might be used to supplement conventional tests for viral infections.

In a previous study we detected serum interferon in all 18 rabbits after subcutaneous infection with a highly virulent strain of PED virus. Sixteen animals showed an A response, two a B response, and peak interferon serum titres of 9 to 243 were attained within 2-3 days.[5] The present 48-hour sera from rabbits infected simultaneously with treponemes and PED virus were therefore considered to be useful for detecting serum interferon. We expected that interferon in serum would correlate with the early phase of PED virus infection in the animals as observed in our infection experiment, but only 27 out of the 39 virus-positive sera showed an interferon titre of >9.

Several factors may have contributed to this discrepancy. The interferon assay was carried out on single serum samples from rabbits of various breeds and possibly these rabbits differed in their ability to produce interferon. The rabbits had received a double infection with treponemes and PED virus, and the sera had been shipped to us under less than adequate conditions for preserving their viral inhibitory effect. The correlation between interferon production and severity of disease in comparable rabbits also suggests that the virulence of the isolates was of some importance in the interferon response and the above discrepancy. This is supported by the results of an infection experiment with an isolate from laboratory No 12 (unpublished observation), in which four rabbits infected subcutaneously gave a C or D clinical response and maximum interferon titres of 9, 3, 3, and <3 on the second or third day. This finding corresponds to the findings with sera from laboratories Nos 12 and 5b.

Clearly, an interferon titre of 3 or transient fever after serum inoculation does not necessarily indicate that the rabbit serum contained PED virus. Two of the 16 postinoculation sera from the virus-negative group showed a rise in titre from <3 to 3 and one serum produced transient fever at inoculation. Similarly, four of the 55 preinoculation sera resulted in transient fever. The interpretation of these findings remains speculative, but non-viral inducers or micro-organisms other than PED virus cannot be excluded. Nevertheless, serum interferon titres of ≥ 9 always indicated the presence of PED virus and, as seen from table II, all laboratories in which PED virus was present had one or more sera with titres of ≥ 9 in contrast to laboratories from whose rabbit sera no PED virus was isolated.

In this study the serum interferon assay provided useful presumptive evidence that PED virus was a contaminant of rabbit-passaged treponemal suspensions. At present PED virus can only be diagnosed by a time-consuming test in rabbits. The interferon assay may therefore be a simple method for detecting possible viral contamination of *T pallidum* propagated in rabbits.

References

1. Gudjónsson H, Newman B, Turner TB. Demonstration of a virus-like agent contaminating material containing the Stockholm substrain of the Nichols pathogenic *Treponema pallidum*. *Br J Vener Dis* 1970;**46**:435-40.
2. Menke HE, van der Heiden CA, Kruizinga W, Kruijt BC, Osterhaus ADME. Intercurrent death of rabbits after inoculation with *Treponema pallidum* in the Netherlands. 7th ICLAS Symposium, Utrecht 1979, Stuttgart: Gustav Fischer Verlag, 1980:263-8.
3. Fennestad KL, Bruun L, Wedø E. Pleural effusion disease agent as passenger of *Treponema pallidum* suspensions from rabbits. Survey of laboratories. *Br J Vener Dis* 1980;**56**:198-203.
4. Gudjónsson H, Newman B, Turner TB. Screening out a virus-like agent from the testicular suspension of the Nichols pathogenic *T pallidum*. With observations on certain characteristics of the agent. *Br J Vener Dis* 1972;**48**:102-7.
5. Fennestad KL, Haahr S, Bruun L. Pleural effusion disease in rabbits. Interferon in body fluids and tissues after experimental infection. *Acta Pathol Microbiol Scand (B)* 1979;**87**:311-5.
6. Small JD, Aurelian L, Squire RA, *et al*. Rabbit cardiomyopathy. Associated with a virus antigenically related to human *Coronavirus* strain 229E. *Am J Pathol* 1979;**95**:709-29.
7. Vaisman A, Paris-Hamelin A, Catalan F, Dunoyer F. Entretien au laboratoire des souches de tréponèmes pathogènes. Precautions à prendre. *La Prophylaxie Sanitaire et Morale* 1973;**45**:71-3.
9. Valle MJ, Jordan GV, Haahr S, Merigan TC. Characteristics of immune interferon produced by human lymphocyte cultures compared to other human interferons. *J Immunol* 1975;**115**:230-3.
10. Fennestad KL, Skovgaard Jensen H-J, Møller S, Bentzon MW. Pleural effusion disease in rabbits. Clinical and postmortem observations. *Acta Pathol Microbiol Scand (B)* 1975;**83**:541-8.
11. Wheelock EF, Sibley WA. Interferon in human serum during clinical viral infections. *Lancet* 1964;ii:382-5.
12. Petralli JK, Merigan TC, Wilbur JR. Circulating interferon after measles vaccination. *N Engl J Med* 1965;**273**:198-201.
13. Jao RL, Wheelock EF, Jackson GG. Production of interferon in volunteers infected with Asian influenza. *J Infect Dis* 1970;**121**:419-26.
14. Matthews THJ, Lawrence MK. Serum interferon assay as a possible test for virus infections of man. *Arch Virol* 1979;**59**:35-8.

Br J Vener Dis 1982; **58**: 147-8

Evaluation of the role of the Treponema pallidum immobilisation test in Britain

M S SPROTT, J B SELKON, AND R H TURNER

From the Regional Public Health Laboratory, Newcastle General Hospital, Newcastle upon Tyne

SUMMARY After specimens of sera from 100 000 patients had been screened by a reagin and *Treponema pallidum* haemagglutination assay (TPHA) 2·7% required further examination. The fluorescent treponemal antibody-absorption (FTA-ABS) test confirmed either a negative or positive TPHA result in 95% of the samples, so that no further investigations were required. This accounted for 99% of the specimens submitted to the laboratory. Repeat tests on a further sample resolved many of the remaining discrepancies. Only 0·05% of patients, in whom repeat tests confirmed a positive TPHA but a negative FTA-ABS result, benefited from a TPI test.

Introduction

Infection with *Treponema pallidum* causes a wide variety of antibodies to be produced. Tests for these fall into two main groups: the first detects the non-specific antitreponemal antibodies, and includes the tests for antilipoidal and treponemal group-reactive antibodies; the second includes the specific *T pallidum* tests. The first work on such a test was described by Nelson and Mayer in 1949,[1] who developed the *Treponema pallidum* immobilisation (TPI) test. This made available a test which was highly specific for treponemes of the syphilis-yaws-bejel group. Its most useful application was considered to be for patients with suspected latent syphilis by differentiating true syphilitic reactions in tests using lipoidal antigen from biological false-positive reactions. The TPI test is technically difficult and was later superseded by the fluorescent treponemal antibody (FTA) test and the more specific FTA-ABS test. In 1965[2] the *Treponema pallidum* haemagglutination assay (TPHA) provided the first specific serological test which could be read with the naked eye.

Which of the specific tests should be used in the diagnosis and monitoring of syphilis is frequently debated. Rein *et al*[3] concluded that the TPI test failed to add important diagnostic information to that obtained by the FTA-ABS test and seriously questioned the need for the former's continued use.

The aim of this study was to determine the value of the TPI test in the serological examination for syphilis in Britain.

Materials and methods

During the period February 1977 to April 1980 specimens of sera from 100 000 patients were submitted to the Public Health Laboratory, Newcastle upon Tyne, for screening for syphilis by the Venereal Disease Research Laboratory (VDRL) and the TPHA tests. Of these sera, 2728 (2·7%) gave positive results in one or other of these tests and were further examined by the FTA-ABS and TPI tests.

In cases in which no clinical information was obtained from the request form submitted with the specimen the physician in charge of the patient was requested to supply relevant details and the ultimate diagnosis.

The Don Whitley TPHA test (Don Whitley Scientific Ltd, Shipley, West Yorkshire) was performed with microtitre equipment according to the manufacturer's instructions using the recommended final serum dilution of 1/80. The TPI, VDRL, and FTA-ABS tests were performed as described[4]; the only notable difference was the use of Wellcome sorbent in the FTA-ABS test at a working titre of 1/5.

Results

The 2728 (2·7%) serum samples giving positive results in either the VDRL or TPHA test or both were examined by the FTA-ABS and TPI tests. The FTA-ABS test confirmed the results of the TPHA

Address for reprints: Dr J B Selkon, Public Health Laboratory, Institute of Pathology, General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE

Accepted for publication 23 November 1981

test in 2611 specimens but discrepancies were found in 117.

TPHA-NEGATIVE/FTA-ABS-POSITIVE RESULTS

Sera from 68 patients gave TPHA-negative and FTA-ABS-positive results and were examined by the TPI test; of these, seven gave positive results and the patients were regarded as having had treponematosis. Of the 61 patients whose sera were TPI-negative, 42 (69%) had clinical evidence of past or recent treponemal disease and 13 (21·3%) had no convincing evidence. For six (9·8%) patients clinical information was inadequate to allow a definitive diagnosis to be made. Thus the TPI test contributed very little to elucidating the discrepancy between sera with negative TPHA and positive FTA-ABS results.

TPHA-POSITIVE/FTA-ABS-NEGATIVE RESULTS

Sera from 49 patients gave TPHA-positive and FTA-ABS-negative results. On examination by the TPI test six (12%) gave positive results (table); four patients had evidence of past or recent treponemal disease but two had none. Of the 43 patients whose sera were TPI-negative, 13 (26%) had evidence of past or present treponemal disease but most (30, 62%) had no clinical or previous serological evidence to suggest the diagnosis of a treponematosis. Nine of these had a second specimen retested and in five all the serological tests including the TPHA gave negative results. Thus, the TPI test again contributed little to elucidating the discrepancy between sera with positive TPHA and negative FTA-ABS results, except that the negative TPI results usually confirmed the diagnosis that the patient did not have a treponematosis.

TABLE *Discrepancies between the TPHA and FTA test results*

Tests	*No of sera*		
	Total reactive	*TPI results*	
		+	–
TPHA – FTA +	68	7	61
TPHA + FTA –	49	6	43
Total	117*	13	104

+ Positive; – negative
*4·3% of 2728 sera
TPHA = *T pallidum* haemagglutination assay; FTA = fluorescent treponemal antibody (test).

Discussion

In this study the discrepancies in which the TPHA test result was negative and that of the FTA-ABS test positive nearly all occurred in patients with early treponemal infection. Since the TPI test is likely to be negative at this stage of the disease its performance adds no additional information and tests repeated on a further specimen would be of greater diagnostic value.

Discrepancies in which the TPHA test result was positive and that of the FTA-ABS test negative did occur in a small number of patients with a past history of a treponematosis. Most patients in this group, however, had no such history or any other evidence of a treponemal infection. In these patients a negative TPI test result was useful supportive evidence of this diagnosis. Some of these patients gave completely negative serological test results when repeat specimens were examined, so that only those patients (estimated at 0·05% of the total population screened) who yielded persistently positive TPHA but negative FTA-ABS test results benefited from additional investigation by the TPI test.

We wish to thank the staff of the serology department for their co-operation in this study.

References

1. Nelson RA, Mayer MM. Immobilization of *Treponema pallidum* in vitro by antibody produced in syphilitic infection. *J Exp Med* 1949;89:369-93.
2. Rathlev T. Haemagglutination test utilising antigens from pathogenic and apathogenic *Treponema pallidum. WHO/VDT/RES/77;*65.
3. Rein MF, Banks GW, Logan LC, *et al.* Failure of the *Treponema pallidum* immobilization test to provide additional diagnostic information about contemporary problem sera. *Sex Transm Dis* 1980;7:101-5.
4. Wilkinson AE. Serological tests for syphilis. In: *Public Health Laboratory Service Monograph Series No 1.* London: HMSO, 1972:1-24.

Br J Vener Dis 1982;**58:**149-50

Syphilis d'emblée due to blood transfusion

Case report

AGUS SOENDJOJO,* MARIANI BOEDISANTOSO,† MOCHAMAD I ILIAS,*
AND DIBJO RAHARDJO*

*From the Departments of *Dermatovenereology and †Internal Medicine, Airlangga University, Surabaya, Indonesia*

SUMMARY A 58-year-old Chinese woman presented with syphilis d'emblée. Seven weeks after a blood transfusion lesions appeared on the palms of her hands and the soles of her feet. These gradually increased in number and other lesions developed on the scalp and palatal mucosa. One of the blood donors was found to have had positive serological test results for syphilis but no clinical symptoms or signs. This case illustrates the danger inherent in the use of whole fresh blood for transfusion purposes.

Introduction

Syphilis d'emblée, or syphilis without a chancre, is a well recognised entity. It occurs as the consequence of direct inoculation of *Treponema pallidum* into the bloodstream, as, for example, in congenital syphilis.[1] Cases of puncture inoculations with infected syringes and tattooer's needles have also been reported.[2 3] Blood transfusion has occasionally been responsible for the transmission of the disease.[1-3] These days the condition is very rare.

Case report

A 58-year-old Chinese married housewife consulted her doctor because of severe anaemia (Hb = 5·6 g/100 ml). She was admitted to hospital in November 1979 and a blood transfusion was given. Seven weeks later, in January 1980, she first noticed macular lesions on the palms of her hands, together with slight fever, headache, anorexia, and arthralgia. She was referred to one of us (AS).

CLINICAL FEATURES

On examination the patient's general condition was good. Her palms and soles showed discrete macular brownish-red lesions 0·5-1 cm in diameter, some of which had white scales at the border. A few moist papular lesions were scattered on the scalp and a mucous patch was present on the hard palatal mucosa. There was no rash on the chest, abdomen, back, or anogenital region. The retroauricular, cervical, and epitrochlear lymph nodes were enlarged, discrete, firm, non-tender, and fully mobile. The inguinal lymph nodes were not palpable.

LABORATORY FINDINGS

The patient was slightly anaemic (Hb = 11 g/100 ml) and had a raised erythrocyte sedimentation rate (Westergren) (33 mm/first hour). No other abnormalities were found.

T pallidum was detected in the moist papular lesions of the scalp by darkfield microscopy. The cardiolipin Wassermann reaction was positive and the serum Venereal Disease Research Laboratory (VDRL) test gave a positive result at a titre of 1/32.

BACKGROUND HISTORY

Marital sexual intercourse had last occurred two weeks before admission to hospital. Her husband, a 60-year-old businessman, had never had genital lesions or a rash in the past and denied extramarital sexual contact within the last three years. His cardiolipin Wassermann and VDRL tests both gave negative results.

The patient had received 2 units of fresh whole blood from four different donors, whose serological tests for syphilis all gave negative results but who had not been physically examined. At our request, the four donors were traced and the tests were repeated. One of the donors was then found to have a reactive cardiolipin Wassermann test and a positive VDRL test result at a titre of 1/16. The donor admitted to sexual contact with a prostitute 10 days before donating blood, but he had not noticed any signs or symptoms of early syphilis, neither of which were evident on physical examination.

Address for reprints: Dr A Soendjojo, Department of Dermatovenereology, Medical Faculty, Airlangga University, Jl Dharmahusada 47, Surabaya, Indonesia

Accepted for publication 11 December 1981

TREATMENT AND FOLLOW UP

The patient was treated with benzathine penicillin G 2·4 million units intramuscularly weekly in two doses. Her post-treatment course was uneventful. All the lesions on the scalp, palms, soles, and palatal mucosa healed within 1-2 weeks after the first injection. Two to three weeks later the lymphadenopathy disappeared. Her serum VDRL titre fell to 1/8 after a month and became negative after 12 months.

Comment

The problem of recognising primary syphilis in women differs greatly from that of identifying primary infection in men. This is because genital lesions in women are often inaccessible and therefore not noticed; also, women are often uninquiring and unaccustomed to seeking medical advice.

This case of syphilis d'emblée illustrates the inherent danger of transfusion with fresh whole blood. Although routine serological tests of donors without clinical examination would detect those in the late primary, secondary, and latent stages of syphilis, they would not exclude those with seronegative primary syphilis or those incubating the disease. Thus, the only safe use of infected blood for transfusion purposes is after storage at 5°C for not less than four days, during which the organisms are presumed to disintegrate.[2]

References

1. Prakken JR. *Textbook of Venereal Diseases*, 2nd ed. Amsterdam: Scheltma and Holkema, 1956.
2. Stokes JH, Beerman H, Ingraham NR, *Modern Clinical Syphilology*, 3rd ed. Philadelphia: W B Saunders Co, 1944.
3. Willcox RR. *A Textbook of Venereal Diseases and Treponematoses*. 2nd ed. London: William Heinemann Medical Books Ltd, 1964.

Br J Vener Dis 1982; **58**: 151-7

Morbidity associated with pelvic inflammatory disease

MICHAEL W ADLER, ELIZABETH H BELSEY, AND BRENDAN H O'CONNOR
From the Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, London

SUMMARY To identify the long-term sequelae and morbidity associated with acute pelvic inflammatory disease (PID) patients with acute PID and matched controls were interviewed at five-monthly intervals for about 21 months after entry into the study. In some instances morbidity among the patients was increased, particularly at five months after admission to hospital. Significantly more patients than controls had visited hospital as outpatients, been admitted to hospital and undergone abdominal operations, and had to alter their normal daily routine and take time off work. The cumulative rates for all of these, except for time off work, were significantly higher in the patients than in the controls. Differences between the two groups both at early and later interviews and cumulatively were evident in the incidence of abdominal pain (other than menstrual pain), change in menstruation (longer and more painful), and pain during sexual intercourse, which persisted in one-fifth of patients after the initial acute episode.

Introduction

The relative ease of cure of most of the sexually transmitted diseases (STDs) detracts from the major contemporary problems associated with them, such as acute and chronic pelvic inflammatory disease (PID), infertility, the possible association with carcinoma of the cervix, and anxiety. PID is the most important complication of gonococcal, chlamydial, and non-specific infections in women. Between 1968 and 1977 the number of patients with acute PID admitted to hospitals in England and Wales rose by 50%[1] and in 1977 reached 10 960, representing 92 000 hospital-bed days at a cost of nearly £7 million (1981 prices). In the United States the direct costs of hospital admissions alone are calculated at nearly US$400 million annually.[2] Furthermore, a much larger number of cases is managed on an ambulant basis, for which no routine statistics are available.

This increase in acute disease is presumably associated with an increase in long-term complications and morbidity. Of these, sterility is the most serious and the best documented, but in addition patients are susceptible to recurrent pelvic pain, dysmenorrhoea, other menstrual disorders, chronic ill health, and can often become "depressed and embittered with life" after an attack of PID.[3-4] Even though these long-term sequelae are recognised, most descriptions are founded on clinical impression. The more scientific studies of patients with PID are based on women returning to hospital, concentrate mainly on the two features of pain and infertility, and often do not use controls. Thus, we decided to undertake a study to describe the morbidity associated with PID in wider terms than hitherto and to compare any changes or events with those observed in a group of controls.

Patients and methods

Female patients aged 15-44 inclusive were included in the study when admitted with presumed acute PID under the care of gynaecologists to one of five hospitals in London (Middlesex Hospital, Soho Hospital, St Mary's Hospital, Samaritan Hospital, and Central Middlesex Hospital).

After discussion with all participating clinicians a check list of clinical diagnostic features was drawn up which took account of their current diagnostic criteria and those used in previous studies.[5-7] These were: constant lower abdominal pain, cervical motion tenderness, adnexal tenderness, uterine tenderness, severe lower abdominal tenderness, purulent vaginal discharge, and pyrexia. All of these were graded by severity and the level of pyrexia measured.

Patients were excluded from the study if they had a previous history of PID, had received any antimicrobial chemotherapy for the current episode or in the previous four weeks, or had undergone a termination of pregnancy, aborted, or given birth in the previous six months.

Address for reprints: Professor M W Adler, Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, London W1N 8AA

Accepted for publication 30 December 1981

BACTERIOLOGICAL SPECIMENS

The doctor admitting any patient considered suitable for the study collected specimens for the isolation of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in a standard manner. Material from the cervix and urethra was inoculated directly on to plates of Columbia blood agar for the isolation of *N gonorrhoeae*. These were transported to our own laboratory for examination. Antichlamydial antibody titres in serum and cervical and urethral fluid may be correlated with infection.[8-9] Specimens were taken from the cervix and urethra with specially designed micro-sponges, sent to the Institute of Ophthalmology, London, and examined by Professor S Darougar.

INITIAL AND FOLLOW-UP INTERVIEWS

Each patient in whom the diagnosis of PID was made was approached by a doctor or nurse from the research team as soon as possible after admission; if the patient agreed to take part in the study an initial interview was carried out with a structured questionnaire. After discharge from hospital, patients were followed up for about 21 months and interviewed at home by a nurse or doctor at five-monthly intervals. Any medical events or changes in social or marital state and sexual intercourse since the previous interview were recorded on a schedule similar to that used at the first interview.

CONTROL GROUP

Each patient in the study was matched with a control by age, marital state, social class, parity, ethnic origin, and method of contraception (intrauterine device or other methods). The control group did not have gynaecological examinations or microbiological tests performed at the beginning of the study. Controls were drawn from women attending family health and planning clinics in the same areas as the hospitals in the study. They were interviewed and followed up in the same way as the patients with PID using the same questionnaire.

INFORMATION COLLECTED

Demographic and medical

At the initial and subsequent interviews basic demographic data and information on past and present medical, obstetric, and menstrual history were recorded; the frequency of sexual intercourse, partner change, and dyspareunia were also noted. At the follow-up interviews both groups were asked whether they had had any illness which had caused them to take time off work or alter their normal daily routine.

Attitudinal and interpersonal

In an attempt to discover underlying attitudinal causes of differences in morbidity between the patients and controls, both groups were asked to complete a semantic differential at each of the five interviews.[10] Each patient was asked to rate certain concepts on a set of seven-point bipolar adjectival scales in order to measure the meaning the concepts had for them. Four concepts were rated: "myself", "my sexual partner", "sexual intercourse", and "work". The first two concepts, rated on sixteen scales, represented general and sexual evaluation and anxiety; the third concept reflected evaluation and anxiety while the fourth represented general evaluation, anxiety, and motivation.

At each interview, a rating of marital adjustment was made by the interviewer for those women in both groups who had a steady partner, either husband or boyfriend. Ratings of sexual and work adjustment were also made. Fuller details of the methods used to obtain these ratings are available elsewhere.[11]

ANALYSIS OF FOLLOW-UP DATA

Life table analysis was the obvious method of choice for comparing morbidity in the two groups. No information on the exact timing of reported events was recorded, however. Although it could have been assumed, for example, that these events had occurred at the mid-point of the interval since the last interview, any such assumptions would have been subject to considerable error. We therefore decided that the cumulative rates of occurrence for each type of event would be compared between the two groups at each follow-up interview. Thus, for instance, the cumulative rate for abdominal pain in the group with PID at the second follow-up interview was found by taking the number of patients who had reported abdominal pain at the first or second follow-up interviews and dividing it by the number of patients who remained in the study to the second follow up. Women who reported abdominal pain at the first follow-up interview, but were lost at the second, were excluded from the calculation of this rate.

All the differences reported between the two groups were statistically significant at the 5% level.

Results

Seventy-eight patients with PID and 77 controls were entered into the study.

PRESENTING FEATURES AND LABORATORY RESULTS

Patients with PID

Lower abdominal pain and adnexal tenderness were the commonest presenting features and were found in over 90% of patients, followed by cervical motion tenderness (81%) and uterine tenderness on bimanual examination (70%). In addition, 31 (40%) women were pyrexial (>37·2°C). Only 8% of women had less than four of the seven presenting features recorded, while over half (51%) had six or all seven.

N gonorrhoeae was isolated from 14 (18%) patients and *C trachomatis* from four (5%). A further 20 patients had one or more raised antichlamydial antibody titres, suggesting an acute infection. The remaining 40 (51%) patients in whom no organisms or raised antibody titres were found were diagnosed clinically as having non-specific PID. The erythrocyte sedimentation rate was raised in 10 of the 28 women for whom it was recorded; the highest rate was 71 mm/first hour. Twenty-eight of the 45 patients in whom a white blood cell count was made had values $>10 \times 10^9$/l.

DEMOGRAPHIC DATA AND MEDICAL HISTORY AT INITIAL INTERVIEW

Patients and controls

The mean age of women with PID was 24·0 years (standard error 0·65), and that of the control group 23·3 years (standard error 0·55). No statistically significant differences were found between the patients and controls in relation to marital state or social class. Significantly more of the patients with PID were of African, Middle Eastern, or mixed origin while fewer were Caucasian. Further analysis, however, showed the ethnic origin was not related to the occurrence of events or changes during the follow-up period.

Sixty-four (82%) women with PID and 68 (88%) women in the control group had regular sexual partners; the mean numbers of sexual partners in the previous year were 2·0 and 1·4 respectively. Patients with PID often reported that they were using no contraceptive method; the controls, however, were significantly more likely to be taking the contraceptive pill.

TABLE I *Number of women interviewed at each follow up and mean length of time between entry and each follow-up interview*

	Patients			*Controls*		
	Interviewed		*Mean time after entry (months ±SE)*	*Interviewed*		*Mean time after entry (months ±SE)*
Follow up	*No*	*%*		*No*	*%*	
First	61	78·2	5·77±0·24	65	84·4	5·94±0·29
Second	56	71·8	11·52±0·33	61	79·2	11·74±0·35
Third	53	67·9	16·85±0·35	61	79·2	16·92±0·44
Fourth	46	59·0	21·33±0·42	53	68·8	21·70±0·39

SE = standard error

No differences were found between the two groups in the occurrence or number of past pregnancies and the mean number of children, miscarriages, stillbirths, and terminations.

As expected, the patients with PID had had significantly more abdominal pain (other than menstrual pain) in the preceding three months than the controls. They were also more likely to describe their periods as heavy and to complain of dysmenorrhoea and dyspareunia in the previous three months. The proportions of women in the two groups who had consulted their general practitioner in the previous three months were similar, 58% for those with PID and 48% for the controls.

FINDINGS ON FOLLOW UP

Follow-up rate

Forty-six (59%) of the 78 women with PID originally entered remained in the study to the end, as did 53 (69%) of the 77 women in the control group (table I). The findings on follow up could have been potentially biased by differences between the women who were lost to follow up and those who were not. Within each group, therefore, women who were interviewed at the fourth follow up were compared with those who were lost by that time in respect to all the variables measured at the initial interview.

In the group with PID, the follow-up rate was related to ethnic origin. It was highest among West Indians (84%) and lowest among women of "other" ethnic origin (29%). This meant, however, that by the time the study was completed the differences in ethnic origin between the group with PID and the control group (described earlier) were no longer statistically significant.

Abdominal operations

Women who had had PID were significantly more likely to have had an abdominal operation during the 21-month follow-up period than women in the control group (table II). The difference was most

TABLE II *Abdominal operations: rates between each interview and cumulative rates for patients and controls*

	Patients (%)	*Controls (%)*	*Significance of difference*
Rates between:			
Entry and 1st follow up	16·4	0·0	P<0·01
1st and 2nd follow up	5·4	4·9	NS
2nd and 3rd follow up	13·2	1·6	P<0·02
3rd and 4th follow up	4·3	0·0	NS
Cumulative rate at:			
1st follow up	16·4	0·0	P<0·01
2nd follow up	19·6	4·9	P<0·05
3rd follow up	30·2	6·6	P<0·01
4th follow up	23·9	5·7	P<0·05

NS = not significant

TABLE III *Abdominal pain: rates between each interview and cumulative rates for patients and controls*

	Patients (%)	*Controls (%)*	*Significance of difference*
Rates between:			
Entry and 1st follow up	55·7	9·2	P<0·001
1st and 2nd follow up	37·5	9·8	P<0·001
2nd and 3rd follow up	37·7	6·6	P<0·001
3rd and 4th follow up	19·6	5·7	NS
Cumulative rate at:			
1st follow up	55·7	9·2	P<0·001
2nd follow up	67·9	14·8	P<0·001
3rd follow up	73·6	18·0	P<0·001
4th follow up	73·9	22·6	P<0·001

NS = not significant

noticeable in the first five months after entry to the study, when 10 (16%) patients had an abdominal operation compared with none of the controls. The commonest operation was laparoscopy, which was carried out on six of the 10 patients during this period. During the whole follow-up period, 22 abdominal operations were performed on the patients with PID, and laparoscopy was still the commonest procedure (10 out of the 22 operations).

By the time they reached the fourth follow-up interview, three-quarters of the patients but less than one-quarter of the controls had reported abdominal pain other than dysmenorrhoea (table III). Reporting rates were higher in the group with PID throughout the follow-up period. At all interviews except the last the patients were also significantly more likely to complain that sexual intercourse had become painful (table IV).

Menstrual history

At each follow up, the women were asked whether they had noticed any change in their periods since the preceding interview and if so to specify what change had occurred. Some women in both groups became pregnant during the study and were therefore excluded from the analysis of menstrual changes. At the first follow up, there was no significant difference between the groups in the proportion of women who reported a change. Thereafter, however, the reporting rates were consistently higher in the group with PID (table V). By the end of the study, 80% of patients but only 44% of controls had said that their periods had changed in some way. The patients were more likely to report longer and more painful periods.

TABLE IV *Pain during sexual intercourse: rates between each interview and cumulative rates for patients and controls*

	Patients (%)	*Controls (%)*	*Significance of difference*
Rates between:			
Entry and 1st follow up	29·1	3·4	P<0·001
1st and 2nd follow up	21·2	1·7	P<0·01
2nd and 3rd follow up	23·4	5·1	P<0·02
3rd and 4th follow up	17·1	7·5	NS
Cumulative rate at:			
1st follow up	29·1	3·4	P<0·001
2nd follow up	37·0	5·0	P<0·001
3rd follow up	43·4	10·0	P<0·001
4th follow up	41·3	13·2	P<0·01

NS = not significant

TABLE V *Change in periods: rates between each interview and cumulative rates for patients and controls*

	Patients (%)	*Controls (%)*	*Significance of difference*
Rates between:			
Entry and 1st follow up	40·8	29·1	NS
1st and 2nd follow up	39·2	12·2	P<0·01
2nd and 3rd follow up	43·1	22·4	P<0·05
3rd and 4th follow up	38·5	19·1	NS
Cumulative rate at:			
1st follow up	40·8	29·1	NS
2nd follow up	60·5	28·6	P<0·01
3rd follow up	74·3	35·7	P<0·01
4th follow up	80·0	44·1	P<0·01

NS = not significant

Obstetric history

By the end of the 21-month follow-up period, 14 (30%) patients and 19 (36%) controls had become pregnant. This difference was not statistically significant. Similarly, there were no significant differences in rates of conception, miscarriage, stillbirth, and ectopic pregnancies between the patients with PID and controls.

Medical consultation

Approximately 50% of women in both groups stated that they had consulted their general practitioner between each interview, up to the third follow up (table VI). Between the third and fourth follow ups, however, 29 (63%) patients had contacted their doctor compared with only 20 (38%) of the controls. When the cumulative rates were examined, paradoxically they were higher in the control group than in the group with PID, with the difference almost reaching statistical significance at the fourth follow up. This indicates that within the group with PID a number of women attended their doctor repeatedly but that within the control group a greater number of women did so on fewer occasions.

Seventy per cent of patients visited a hospital between their initial and the first follow-up interviews compared with only 20% of controls, but no differ-

TABLE VI *Consultation with general practitioner: rates between each interview and cumulative rates for patients and controls*

	Patients (%)	*Controls (%)*	*Significance of difference*
Rates between:			
Entry and 1st follow up	50·8	50·8	NS
1st and 2nd follow up	44·6	54·1	NS
2nd and 3rd follow up	52·8	44·3	NS
3rd and 4th follow up	63·0	37·7	$P<0·05$
Cumulative rate at:			
1st follow up	50·8	50·8	NS
2nd follow up	62·5	77·0	NS
3rd follow up	79·2	88·5	NS
4th follow up	87·0	96·2	NS

NS = not significant

ences in hospital visiting rates between the groups were found thereafter. Similarly, more patients (20%) than controls (6%) were admitted to hospital in the first five months of the follow-up period. The commonest reason for admission was abdominal pain (eight of the 12 admissions). The cumulative rate for hospital admission was significantly higher in the group with PID than in the control group at the fourth follow up (54% compared with 28%). Again, the commonest cause of admission was abdominal pain. This apparent difference may well have been caused by the loss to follow up of women who had been admitted to hospital in the control group rather than by a genuine difference between the groups.

Change in daily routine

Women in both groups were asked whether they had had to alter their normal day-to-day routine because of any illness they had had since their previous interview. In the first five months after the start of the study, 17 (28%) patients but only two (3%) controls stated that they had had to do so. The cumulative rates were significantly higher in the group with PID throughout the follow-up period (table VII). By the fourth follow-up interview, 59% of patients had reported a change in their routine because of illness compared with only 15% of controls.

TABLE VII *Alteration of normal daily routine because of illness: rates between each interview and cumulative rates for patients and controls*

	Patients (%)	*Controls (%)*	*Significance of difference*
Rates between:			
Entry and 1st follow up	27·9	3·1	$P<0·001$
1st and 2nd follow up	16·1	4·9	NS
2nd and 3rd follow up	7·5	4·9	NS
3rd and 4th follow up	28·3	11·3	NS
Cumulative rate at:			
1st follow up	27·9	3·1	$P<0·001$
2nd follow up	33·9	6·6	$P<0·001$
3rd follow up	37·7	9·8	$P<0·001$
4th follow up	58·7	15·1	$P<0·001$

NS = not significant

Between the initial and the first follow-up interviews, 13 (21%) patients said that they had had to take time off work compared with only two (3%) controls; 15% of women with PID but only 2% of controls said that they had had to stay in bed. Between the first and second follow-up interviews, 16% of patients said they had had to stay in bed compared with 3% of women in the control group. All of these differences were statistically significant.

SEMANTIC DIFFERENTIAL SCORES AND ADJUSTMENT RATINGS

There were no consistent differences between the patients and controls in relation to group mean scores for each dimension of attitude towards the four concepts. The adjustment ratings also differed little between the groups. At the first follow-up interview, 93% of the controls had their marital adjustment rated as satisfactory compared with only 69% of the patients. Ratings of sexual adjustment were higher among the controls at the initial and the first follow-up interviews, but the groups did not differ thereafter. Satisfactory work adjustment was recorded for 82% of women with PID at the third follow up but for 57 (95%) of the 60 controls.

Discussion

Pelvic inflammatory disease can have profound physical and psychological effects on patients, a number of which have been measured in this study. In some instances similarities were evident for both patients with PID and the controls; in others, some differences were seen immediately after discharge from hospital (but they did not persist) and others continued for longer.

Infertility, pregnancy wastage, and ectopic pregnancy are recognised consequences of PID.[12-15] This study did not show any variation between patients with PID and the controls in their ability to become pregnant or the likelihood of their having a miscarriage or ectopic pregnancy by the end of the 21-month follow-up period. Substantial differences have been found, with periods of follow up ranging from 4-9·5 years.[3 12 16] Compared with these studies, our follow-up time was extremely short and probably too short after the acute episode to show any variation which might have existed. In particular, a short follow-up period may militate against the finding of any dissimilarities, since patients may decide to be advised to postpone attempts to become pregnant voluntarily until "things have settled down".

In some instances the differences between patients and controls were most pronounced at the first follow-up interview at 5-6 months after entry. Thus, significantly more patients than controls had visited hospital as outpatients, been admitted to hospital, and had undergone abdominal operations. These findings are not surprising since patients tend to be followed up and observed most intensely immediately after being in hospital, and any persistence of symptoms may be investigated, especially by laparoscopy, as occurred in this study. Significant differences were also found between patients and controls in relation to alteration of their normal daily routine and time off work at the first interview but not thereafter. Again, this is not an unexpected finding since patients will be convalescing after their discharge from hospital.

The cumulative rates for outpatient visits, hospital admissions, abdominal operations, and alteration of daily routine were significantly higher in the group with PID than in the controls. Loss to follow up among the controls may have accounted for some of these variations. Even so, the fact that 89% of patients were seen as outpatients, 54% were admitted to hospital, and 24% underwent abdominal operations represents a substantial cost to the National Health Service. Similarly, the alteration of normal daily routine in 59% of patients during the course of the study is a cost to the individual and to the nation. The economic consequences of illness and treatment are not confined to the Health Service; the patient's household can be affected economically in a variety of ways during admission to hospital and after discharge.

Some of the features measured showed differences between the patients and controls both at early and later follow-up interviews and cumulatively. These were abdominal pain (other than period pain), changes in menstruation (longer and more painful), and pain on sexual intercourse; the cumulative rates for each in patients with PID were 74%, 80%, and 41% respectively. Weström[3] has reported a lower rate for abdominal pain after PID; he indicated that 18% of patients had pain lasting for longer than six months causing them to seek medical advice, while Falk[5] reported that 17% of patients had chronic pain. Our figures were substantially higher. This may be because we were measuring pain which was not necessarily taken to a doctor and was thus less severe; furthermore, patients were never asked whether they had had pain for a specific length of time, such as six months. We could not assess the degree of discomfort experienced by the patients with abdominal pain or the extent to which it impinged on their lives. A proportion of the patients with PID were readmitted to hospital, usually because of abdominal pain, and the commonest procedure was laparoscopy. Continuing pain which requires admission to hospital presents a substantial diagnostic clinical problem. Over 10 000 women a year have laparoscopic examination for abdominal pain.[17]

Increased pain associated with periods was also experienced by the patients with PID, and the reporting rates for menstrual changes after PID were higher in the patients than in the controls throughout the follow-up period. Again the degree of discomfort and disruption of "normal" living cannot be judged, but some patients will require medication and be forced to alter their normal routines.

The final difference between the patients and controls concerned dyspareunia. This feature of PID is not well documented. The degree of disruption that it causes cannot be judged, even though it continued in one-fifth of patients after the initial acute disease episode. Although both sexual and marital adjustment were reduced in the patients at the first follow up, this did not persist, and no major attitudinal differences in the concepts "my sexual partner" and "sexual intercourse" were detected by use of the semantic differential. This may mean either that dyspareunia was not severe enough to interfere with satisfactory sexual relationships or that patients learnt to tolerate their pain without complaining.

The increase in acute PID and the related chronic sequelae mean that this condition has now replaced syphilis as the STD with the most serious long-term consequences.

The study was supported as part of a research contract from the Medical Research Council. We wish to thank all the clinicians and patients who took part. We are particularly grateful to our two research nurses (Julia Vaughan-Smith and Jasmine Peel), who carried out the interviews, and to Professor S Darougar for examining the chlamydial specimens.

References

1. Adler MW. Trends for gonorrhoea and pelvic inflammatory disease in England and Wales and for gonorrhoea in a defined population. *Am J Obstet Gynecol* 1980; **138**: 901.
2. Curran JW. Economic consequences of pelvic inflammatory disease in the United States. *Am J Obstet Gynecol* 1980; **138**: 848.
3. Weström L. Effect of acute pelvic inflammatory disease on fertility. *Am J Obstet Gynecol* 1975; **121**: 707.
4. King A, Nicol C. *Venereal Diseases*. 3rd edition. London: Baillière Tindall, 1975: 207.
5. Falk V. Treatment of acute non-tuberculous salpingitis with antibiotics alone and in combination with glucocorticoids. A prospective double-blind controlled study of the clinical course and prognosis. *Acta Obstet Gynecol Scand* 1965; **44** suppl 6: 3.
6. Wright NH, Laemmle S. Acute pelvic inflammatory disease in an indigent population. *Am J Obstet Gynecol* 1968; **101**: 979.

7. Targum SD, Wright NH. Association of the intrauterine device and pelvic inflammatory disease: a retrospective pilot study. *Am J Epidemiol* 1974; **100**: 262.
8. Treharne JD, Dines RJ, Darougar S. Serological responses to chlamydial ocular and genital infections in the United Kingdom and Middle East. In *Non-gonococcal Urethritis and Related Infections*. Hobson D, Holmes KK, eds. Washington DC: American Society of Microbiology, 1977; 249.
9. Treharne JD, Darougar S, Simmons PH, Thin RN. Rapid diagnosis of chlamydial infection of the cervix. *Br J Vener Dis* 1978; **54**: 404.
10. Osgood CE. *The Measurement of Meaning*. Urbana: University of Illinois Press, 1957.
11. Greer HS, Lal S, Lewis SC, Belsey EM, Beard RW. Psychosocial consequences of therapeutic abortion. *Br J Psychiatry* 1976; **128**: 74.
12. Hedberg E, Speyz SO. Acute salpingitis. Views on prognosis and treatment. *Acta Obstet Gynecol Scand* 1958; **37**: 131.
13. World Health Organisation. The epidemiology of infertility. *Technical Report Series 582*. Geneva: WHO, 1975.
14. Weström L, Iosif S, Svensson L, Mårdh P-A. Infertility after acute salpingitis: Results of treatment with different antibiotics. In: *Sexually Transmitted Disease Symposium Proceedings*. New York: Science and Medicine Publishing Co, 1979: 5.
15. Weström L, Mårdh P-A. Reproductive events after acute salpingitis. In: *Swedish Studies on Infertility and Ectopic Pregnancy*. Washington DC: National Institute of Health, 1980.
16. Holtz F. Klinische studien uber die nicht tuberkulose salpingoloophritis. *Acta Obstet Gynecol Scand* 1930; **10** suppl 1.
17. Working Party of the Confidential Enquiry into Gynaecological Laparoscopy. Gynaecological laparoscopy. *Br J Obstet Gynaecol* 1978; **85**: 401.

Br J Vener Dis 1982;**58**:158-65

Neisseria gonorrhoeae isolated from disseminated and localised infections in pre-penicillin era

Auxotypes and antibacterial drug resistances

B WESLEY CATLIN* AND ALICE REYN†

*From the *Department of Microbiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA; and †Vesterled 12, Copenhagen, Denmark*

SUMMARY Interest in the evolution of gonococcal auxotrophy led to a study of 72 strains isolated between 1935 and 1948 from the urogenital tract (57 patients), the eye (two patients), and from disseminated gonococcal infections (11 patients and probably two others). Two cervical isolates with nutritional requirements for proline, arginine, histidine, and biotin were oxidase-positive, Gram-negative diplococci, but their identity as *Neisseria gonorrhoeae* was uncertain because they were atypically susceptible to colistin and did not produce acid in glucose media. The *N gonorrhoeae* strains were highly susceptible to 11 other antibacterial drugs but not to sulphadiazine. Defects of one or more pathways for the biosynthesis of methionine, proline, arginine, threonine, lysine, the branched-chain amino acids, hypoxanthine, and thiamine pyrophosphate were found in 39 of the 70 strains, including four isolated in the pre-sulphanilamide era. Unexpectedly, methionine was required for the growth of 11 (21%) of the 52 Danish strains and for 13 (72%) of 18 strains isolated in the USA. The Danish strains included 28 (54%) that did not require any of the compounds used for differentiating auxotypes, whereas this type was represented by only three (17%) of the USA strains. None of the gonococci required uracil or other pyrimidines. This suggests that the requirements for arginine, hypoxanthine, and uracil commonly found in recent isolates from disseminated gonococcal infections probably evolved after treatment with sulphonamide was replaced by penicillin.

Introduction

The development of gonococcal resistances to antibacterial drugs over the past four decades is well known.[1-4] In contrast, little is known about the evolution of the nutritional requirements of *Neisseria gonorrhoeae*. Gonococci isolated in the 1970s require a variety of compounds for growth in chemically defined media.[5] Some components, such as cysteine or cystine, are required by all members of the species. Various other compounds are required by some gonococcal isolates but not by all. This diversity of nutritional requirements differentiates between *N gonorrhoeae* isolates by a system of auxotyping.[6] The accompanying study[7] characterises strains isolated in 1975 from patients in one community and cites other auxotyping studies.

N gonorrhoeae strains with requirements for compounds that include arginine, hypoxanthine, and uracil have attracted special attention because of their propensity for invading the bloodstream.[8-10] These gonococci, called AHU strains by Knapp and Holmes,[8] are widely prevalent[11-13] and increasing in some communities.[10] A peculiar feature of AHU strains is their uniform susceptibility to penicillins, streptomycin, and most other antibiotics; this occurs even in areas where many other isolates of *N gonorrhoeae* are relatively resistant. Further studies of gonococci from disseminated infections indicated that penicillin susceptibility was more notably related to dissemination than was the AHU pattern of nutritional requirements.[10 14] Present information suggests that the AHU pattern is best regarded as an indicator of gonococci which may possess one or more properties conducive to dissemination.

Treatment of patients with antibiotics in doses that are only partially effective is recognised as a form of natural selection which has resulted in the decreased susceptibility of gonococci to various drugs. Never-

Address for reprints: Professor B Wesley Catlin, Department of Microbiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226, USA

Accepted for publication 23 December 1981

theless, the possible effects of selection on auxotrophy remain obscure. An important question concerns the prevalence of AHU strains and other auxotrophic gonococci before the introduction of penicillin in the mid-1940s. Since *N gonorrhoeae* strains isolated between 1935 and 1948 from patients with disseminated or localised infections are available, we have been able to examine the nutritional requirements of gonococci isolated before the penicillin era and to obtain baseline data on their susceptibilities to various antibacterial drugs with differing mechanisms of action.

Materials and methods

The methods described in the accompanying study[7] were used to determine the nutritional requirements and responses to 13 antibacterial drugs of 72 strains of *N gonorrhoeae* isolated more than 30 years ago. These bacteria were preserved by freeze-drying in glass ampoules.

COLLECTIONS OF GONOCOCCI

Eighteen strains of *N gonorrhoeae* from the collection of Sara Branham[15] were obtained through the kind co-operation of Carl E Frasch (Bureau of Biologics, Food and Drug Administration, Bethesda, Md, USA; table I).

Strains from Danish patients were obtained from the collection held by the *Neisseria* Department of the Statens Seruminstitut, Copenhagen, Denmark. Detailed examinations were made of 97 isolates from urogenital specimens. Some isolates, however, were related, being from two anatomical sites, or repeated cultures from a given patient, or cultures from marital partners. The related isolates are regarded as representatives of one strain since they resemble one another in all cases except two, which are described below.

The Statens Seruminstitut designations of the 54 strains of this study group are listed below with the total number of related isolates where more than one representative of a given strain was studied (in parentheses). Eight strains were isolated in 1940: 17792, 17941 (2), 17932 (2), 18000 (2), 18218 (2), 18887 (2), 19832, and 20514 (2). Four strains were isolated in 1941: 23363 (2), 31956, 32328 (2), and 32423 (3). Ten strains date from 1942: 50004, 50601, 51768 (2), 65171 (2), 65983 (5), 65988 (4), 66179 (3), 66375, 68808, and 69016 (2). The following 27 strains were isolated in 1943: 23157, 23232, 25590, 26509, 26876 (2), 28466, 29098, 29654, 30517 (2), 40635 (3), 40914 (2), 41835 (3), 41842, 42809 (2), 43467, 44353 (2), 45045, 45689, 46269 (2), 46473 (2), 46615 (2), 47750 (3), 50123, 50602 (2), 58449 (2), 61350 (2), and 64683. Strains 65479 (3) and 65485 (3) were isolated in 1944. Sulphadiazine-resistant strains 7764/45, W-18/46, and W-58/46, isolated in Copenhagen in 1945 and 1946, were described earlier[5 6] and studied in more detail here.

The lyophilised cultures were revived on multiple plates of GCMBS agar[7] supplemented with rabbit blood or sheep blood. Subcultures were made routinely on GCMBS agar. The identification methods given earlier[16 17] were used to determine the species.

NUTRITIONAL REQUIREMENTS

The standard NEDA media were used for auxotyping.[7 17] All strains required cysteine or cystine for growth. A strain which grew on all other auxotyping

TABLE I *Origins and auxotypes of 18 strains of Neisseria gonorrhoeae isolated in the USA, 1935-48*

Patient					
Site	*Sex*	*Locality*	*Month/year*	*Strain*	*Auxotype*
CSF	F	Hartford, CT	7/35	*G*-2	Met-Lys
Unknown		York, PA	6/36	*G*-4	Pro-Met
CSF		Memphis, TN	10/36	*G*-5	Met*
Eye		Rochester, NY	12/36	*G*-7	Zero
Urine		Rochester, NY	1/37	*G*-9	Zero
Eye		Rochester, NY	1/37	*G*-10	Pro
CSF	F	Washington, DC	9/37	*G*-11	Pro-Arg-Met*†
CSF	F	Washington, DC	11/37	*G*-12	Arg-Met*
CSF	M	Washington, DC	11/37	*G*-14	Arg-Met*-Ile
Blood	M	Washington, DC	3/38	*G*-15	Met
CSF		Valhalla, NY	10/38	*G*-17	Met*
Blood		Baltimore, MD	5/39	*G*-20	Pro
Urethra	M	Baltimore, MD	5/39	*G*-22	Pro-Met
Urethra	M	Baltimore, MD	10/37	*G*-23	Zero
Blood	M	Washington, DC	6/39	*G*-24	Met*
Unknown		Fort McPherson, GA	4/45	*G*-26	Pro-Met-Leu-Hyx
CSF	M	Washington, DC		*G*-27	Met*
Blood	F	Washington, DC	4/48	*G*-28	Arg-Met*-Thr

*Requirement for methionine is satisfied by homocysteine but not cystathionine

†Auxotype of *G*11-1 and seven other spontaneous mutants independently derived from the parent strain *G*-11 which does not grow on the auxotyping media

UNIVERSITY COLLEG LIBRA

media was classified in the Zero auxotype. The designations for other auxotypes include one or more of the following: Pro (proline), Arg (arginine), Met (methionine), Hyx (hypoxanthine), Thp (thiamine pyrophosphate), Lys (lysine), Leu (leucine), Ile (isoleucine), Thr (threonine), His (histidine), and Bio (biotin).

An auxotype is a category in which gonococci are classified according to their pattern of nutritional requirements. Auxotypes do not carry superscripts[17]; however, a superscript is used when referring to a particular nutritional trait displayed by bacteria, such as an arginine requirement (Arg^{-}) or non-requirement (Arg^{+}).

DETERMINATION OF RESISTANCE

GCMBS agar was the basal medium used for tests of the antibacterial activities of various concentrations of 13 drugs. The benzylpenicillin used in this study was potassium penicillin G (control No W653905, potency 1580 IU/mg, generously supplied by Wyeth Laboratories Inc, West Chester, Pennsylvania). Spectinomycin sulphate was U-18409E (potency 641 μg/ml, generously supplied by the Upjohn Company, Kalamazoo, Michigan). Other antibiotics, the test method, and the end-point assigned for resistance were the same as before.[7]

Results

The 18 strains listed in table I and 50 Danish strains (table II) were typical representatives of *N gonorrhoeae*. Negative reactions in glucose tests were found, however, for the two Pro-Arg-His-Bio strains and for two Zero auxotype strains, *SS*50004/42 and *SS*51768/42 (and the related isolate *SS*51769/42). Acidity was not detected in repeated tests performed either on a growth medium or in Elrod-Braun buffered salts solution containing 1% or 10% glucose.[16] All strains, including these four, were oxidase-positive and required cysteine or cystine. The Zero auxotype strains appeared to be gonococci which were atypical only with respect to the glucose reaction; their antibiotic resistances were similar to those of the typical gonococci. The identification of the Pro-Arg-His-Bio strains was less certain and most of their resistances differed from the mean values for the 70 strains; consequently, the resistance levels for these two were not included in table III. It is not known whether the Pro-Arg-His-Bio strains, *SS*68808/42 and *SS*25590/43, came from the same or different patients, but each was regarded as a case of gonorrhoea. Two different municipal outpatient clinics obtained the cultures, one in October 1942 from a patient described as "female, 23 years" and the other in June 1943 from a "female, 24 years." Both strains were oxidase-positive, Gram-negative diplococci and their inability to acidify glucose, maltose, sucrose, or lactose might allow them to be classified as *Branhamella catarrhalis*[18] or *Neisseria flavescens*[19] rather than as atypical *N gonorrhoeae*. Studies of the genetic relationship to these species are needed for accurate identification.

TABLE II *Auxotypes of the 72 N gonorrhoeae strains isolated, 1935-48*

Auxotype	*No of strains*	
	Denmark	*USA*
Zero	28	3
Pro	3	2
Arg	3	0
Met	3	5
Hyx	1	0
Thp	2	0
Pro-Arg	1	0
Pro-Met	3	2
Pro-Thp	3	0
Arg-Met	2	1
Met-Thp	2	0
Met-Lys	0	1
Pro-Arg-Met	0	1
Arg-Met-Ile	0	1
Arg-Met-Thr	0	1
Pro-Arg-His-Bio	2*	0
Pro-Met-Ile-Thp	1	0
Pro-Met-Leu-Hyx	0	1

*Identity as *N gonorrhoeae* uncertain

NUTRITIONAL REQUIREMENTS

The most notable findings were the large number of strains (33%) which required methionine (table II) and the absence of AHU strains even among the gonococci isolated from disseminated infections (table I). Pyrimidine biosynthesis was normal in all strains as shown by their growth on media lacking uracil or other pyrimidines. Also, arginine biosynthesis was normal in the two strains which required hypoxanthine, *G*-26 and *SS*40914/43 (together with the related isolate *SS*39781/43).

The diversity of auxotypes was unexpectedly large; 18 auxotypes were found (table II) compared with 14 in the first study.[7] Furthermore, additional types could be obtained by classifying Thp^{-}, Met^{-}, and Arg^{-} strains in auxotypes subdivided to indicate the ability or inability of the gonococci to utilise particular precursor compounds for growth. Thus, appreciable growth of seven Thp^{-} strains occurred only on a medium containing thiamine pyrophosphate, whereas thiamine partially satisfied the requirement of one strain listed as Thp (*SS*30517/43 and the related isolate *SS*30778/43). Of the 24 Met^{-} strains (table II), eight listed in table I multiplied on a medium in which methionine was replaced by homocysteine, the immediate precursor in this biosynthetic pathway.[20]

The 12 Arg$^-$ strains bore one or more lesions of three different gene loci (*arg*A, *arg*E, and *arg*G) that encode enzymes needed for arginine biosynthesis. (a) The Pro-Arg strain (*SS*23157/43) and one Arg-Met strain (represented by isolates *SS*46269/43 and *SS*46459/43) could grow if arginine was replaced by any one of the following intermediate compounds: N-acetylglutamate, N-acetylornithine, ornithine, citrulline, or argininosuccinate. This indicated that most of the arginine biosynthetic steps were operative and suggested that the enzyme which synthesises N-acetylglutamate (encoded by *arg*A) was defective.[20 21] (b) Nine of the other Arg$^-$ strains could utilise ornithine, citrulline, or argininosuccinate for growth but not the acetylated precursors (*arg*E lesions).[21 22] (c) The arginine requirement of *G*11-1 was satisfied by argininosuccinate but not by any of the other precursor compounds listed (*arg*G lesion). Strain *G*11-1 also bore a mutant *arg*E gene locus, which was detected accidently when the locus was segregated by genetic transformation. Treatment of an Arg$^+$ recipient with *G*11-1 DNA produced a transformant which was Arg$^-$ and able to utilise citrulline and ornithine but not N-acetylornithine.

Strain *G*-11 (table I) was an isolate from cerebrospinal fluid from a patient (BM) described by Branham and colleagues.[15] Growth of the parent strain *G*-11 was fastidious on complex media and extremely limited on the defined media. The nature of this limitation, whether due to an unsatisfied nutritional requirement or to inhibition,[17] is not yet known. Every large population of *G*-11 cells, however, contained a few mutants, which formed colonies on NEDA media. *G*11-1 and seven other spontaneous mutants have been examined and found to be identical in their nutritional requirements, and their resistance profiles were similar to one another and to the parent *G*-11. In addition to strict requirements for proline, arginine, and methionine, the mutants were stimulated by thiamine pyrophosphate. Growth does not occur on NEDA lacking leucine, isoleucine, and valine or on media containing any one or two of these amino acids. Because the biosynthetic pathways of the branched-chain amino acids are complex and interrelated,[20] the precise requirements remain to be determined.

Thirty-one of the strains were represented by two or more isolates, all but one having been cultured from sexual partners or from different specimens from the same patient. For 29 strains similar auxotypes and resistances showed that the processes of isolation, lyophilisation, storage for 35 years, reconstitution, and further subculturing had not produced notable differences between the related isolates. In two instances concerning the Met-Thp auxotype, however, a nutritional requirement was lost, presumably owing to genetic instability; only the double auxotrophs were listed. The primary culture of the Met$^-$ Thp$^-$ strain *SS*65171/42 produced "fine growth", but after two subcultures larger colonies were observed among the small ones. A large-colony isolate which was preserved in 1942 was Met$^-$ Thp$^+$ and represented a mutant selected for improved growth on a medium presumably containing a suboptimal concentration of thiamine pyrophosphate. The second instance of deviation concerned cultures from two different urethral specimens from one man: *SS*17941/40 was Met$^-$ Thp$^-$ and the other isolate was Thp$^-$ but Met$^+$.

ANTIBACTERIAL DRUG RESISTANCES

The levels of resistance to 12 drugs for 70 of the pre-1950 strains are given in table III. All 70 were resistant to colistin at a concentration $\geqslant$25 μg/ml, 25 strains were resistant to 50 μg/ml and 23 to at least 75 μg/ml. The drug responses of the two Pro-Arg-His-Bio strains were omitted from table III. Their colistin resistance was limited to 0·1 μg/ml in contrast to the resistance of typical gonococci.[23] Also, these two strains were more resistant than most of the 70 strains to benzylpenicillin (0·05 U/ml), oxacillin (0·5 μg/ml), erythromycin (0·5 and 1·5 μg/ml), spectinomycin (15 and 20 μg/ml), vancomycin (40 and 30 μg/ml), and lincomycin (80 and 50 μg/ml). Their resistances to the remaining six drugs fell within the ranges given in table III.

The wide range of resistance to sulphadiazine (table III) was similar to the response to sulphathiazole reported for strains isolated in 1960.[24] On the other hand, the high susceptibilities of the 70 strains to benzylpenicillin and most of the other drugs was typical of bacteria with no previous exposure to these selective agents.[1 24] Compared with the Danish strains, however, the gonococci isolated in the USA showed greater diversity of drug responses. Thus, the five strains resistant to oxacillin at a concentration of 0·5 μg/ml were *G*-5, *G*-15, *G*-17, *G*-24, and *G*-26. *G*-15 and *G*-26 were also resistant to erythromycin at 1·0 μg/ml and *G*-26 to rifampin at 0·5 μg/ml. The gonococci that were most susceptible to antibiotics included *G*-11, *G*-23, and *SS*50602/43 with resistance to benzylpenicillin limited to 0·005 U/ml and oxacillin to 0·05 μg/ml. The resistance to tetracycline of *G*-17 and *G*-23 was 0·02 μg/ml and the chloramphenicol resistance of *G*-11 was 0·1 μg/ml. The resistance of 69 of the 70 strains (table III) was distinctly greater to oxacillin than to benzylpenicillin as expected.[7] The exception was the Zero auxotype strain *SS*45689/43 which was resistant to oxacillin at a concentration of 0·05 μg/ml and to benzyl-

TABLE III *Responses of 70 N gonorrhoeae strains to the antibacterial actions of 12 drugs*

	No of strains with indicated resistance:*									
Antibacterial drug	*0·005*	*0·01*	*0·02*	*0·05*	*0·1*	*0·2*	*0·5*	*1·0*	*1·5*	*2·0*
Benzylpenicillin	4	59	7	0	0	0				
Oxacillin		0	0	4	18	43	5	0		
Tetracycline		0	2	19	48	1	0			
Chloramphenicol			0	0	2	68	0			
Erythromycin		0	0	1	30	36	1	2	0	0
Rifampin		0	20	47	2	0	1	0		
					0·1	*0·2*	*0·4*	*0·6*		
Nalidixic acid					0	39	31	0		
	0·5	*1·0*	*2·0*	*5*	*10*	*15*	*20*	*30*		
Streptomycin	0	2	18	50	0	0				
Spectinomycin		0	1	12	51	6	0	0		
	1·0	*2·0*	*3·0*	*5*	*10*	*20*	*30*	*40*		
Vancomycin	0	1	5	20	31	12	1	0		
Lincomycin			0	2	19	39	10	0		
	0·2	*0·5*	*1·0*	*2·0*	*5*	*10*	*20*	*35*	*50*	*≥100*
Sulphadiazine	5	14	9	11	3	15	5	3	3	2

*Expressed as the highest concentration of drug permitting growth; concentrations of benzylpenicillin in IU/ml all other drugs in μg/ml.

penicillin at 0·01 U/ml. This strain was inhibited by vancomycin at 3 μg/ml in the standard test, and smaller inocula were more susceptible, which suggests that these gonococci would not have been recovered on the vancomycin-containing media now used for primary isolation.[7]

Discussion

Disseminated gonococcal infections were caused by 11 of the 18 strains listed in table I and probably also by strains *G*-4 (referred from a sanitorium) and *G*-26 (from a US army medical laboratory). It is remarkable that although all 13 strains possessed one or more auxotrophic traits, none required uracil. The Zero auxotype accounted for only one-sixth of these 18 isolates compared with more than half of the Danish strains (table II), all of which were from urogenital sites. Defects of one or more pathways for biosynthesis of proline, arginine, methionine, threonine, lysine, the branched-chain amino acids, hypoxanthine, and thiamine pyrophosphate were found in 39 of the 70 *N gonorrhoeae* strains. A nutritional requirement for glutamine was not detected, although it was common at that time in gonococci isolated in Texas[25] where thiamine pyrophosphate-requiring strains were also found.[26] The unexpected variety of biosynthetic defects indicated that these nutritional traits have evolved for a very long time.

Successful treatment of gonorrhoea with penicillin was reported in 1943,[27] but Fleming[28] and Harkness[29] noted in 1944 that the limited supply prohibited its use for treating venereal diseases. Perdrup[30] gave 1947 as the year penicillin was becoming widely used in Denmark. Since more than 60 of the *N gonorrhoeae* strains listed in tables II and III were isolated before 1944, we must look to other agents as potential sources of selection of auxotrophic mutants.

Gonococci have been exposed to many compounds because patients often had both syphilis and gonorrhoea. Urethral irrigations with potassium permanganate,[29] organic preparations of silver,[31 32] or proflavin[32] were commonly prescribed for gonorrhoea. The therapeutic value of arsphenamine in syphilis was described by Ehrlich in 1910.[33] This compound and other organic arsenicals, together with bismuth, were in use for more than 30 years.[31-33] Older remedies for syphilis listed by Magian[32] were mercury compounds, iodides, and preparations of gold, silver, platinum, antimony, zinc, copper, thallium, iron, and manganese. The bacteriostatic action of the salts of heavy metals is well established and ferrous and manganous compounds are known bacterial mutagens.[34] Nevertheless, beyond recognising the potential effects of these many chemicals on gonococci in patients' tissues, it is not possible now to assess their contribution to the evolution of auxotrophy. On the other hand, a causal relation can be shown between sulphonamide resistance and a requirement for methionine.

The first use of sulphanilamide for treating gonorrhoea, according to Harkness,[29] was by Dees and Colston in 1937.[35] Other more active sulphonamides were soon synthesised, but within 10 years treatment failed in most cases.[24] The rapid development of resistance was aided by challenging the gonococci with subcurative doses of sulphonamides, for example, by self-treatment,[36] by prophylactic use to control respiratory disease,[37] or during successful treatment of streptococcal disease. Furthermore, there was no consensus on many important features of sulphonamide treatment even by 1939.[33]

Twenty per cent of *N gonorrhoeae* isolates from Danish patients in 1940 were sulphonamide-resistant and approximately 40% were resistant in 1944.[24] All 11 of the Danish Met⁻ strains (table II) showed sulphadiazine resistance of ⩾10 μg/ml and all but one of the eight strains with a resistance of ⩾35 μg/ml (table III) required methionine. The Zero auxotype strain *SS*50602/43 was resistant to 35 μg/ml, as were strains *SS*7764/45 (Pro-Met-Thp) and *G*-11 (table I). Resistance to a sulphadiazine concentration of 50 μg/ml was shown by strains *SS*58449/43 (Met), *G*-22, and *G*-26. Resistance of ⩾100 μg/ml characterised strains *SSW*-18/46 (Pro-Met) and *SSW*-58/46 (Met). It is important to point out, however, that the sulphadiazine resistance of the Met⁻ strains *G*-2 and *G*-4 was limited to 1 μg/ml. Both *G*-2 and *G*-4 were isolated in the presulphonamide era. Their defects, as well as those of the 11 Danish Met⁻ strains, however, apparently involved the final step of methionine biosynthesis.

A further study has examined spontaneous sulphadiazine-resistant (Sul-r) mutants selected for increased resistance by growth on sulphadiazine-containing GCMBS agar. Strain *SS*41835/43 and several other Met⁺ sulphadiazine-susceptible (Sul-s) *N gonorrhoeae* strains gave rise to Sul-r colonies which were picked, purified, and auxotyped. Nearly half of the Sul-r mutants were Met⁻; the others remained Met⁺. Homocysteine did not satisfy the requirement of any of these Met⁻ mutants, indicating that the terminal step in methionine biosynthesis was affected. Many of the mutations were, however, distinguishable by genetic transformation.

Transformation mediated by deoxyribonucleic acid (DNA) had earlier shown the non-identity of the mutations borne by several Sul-r isolates from patients and suggested the relationship between sulphonamide resistance and methionine auxotrophy.[38] Separate exposures of a Sul-s Met⁺ recipient to DNA preparations from two Sul-r Met⁻ strains (*SS*7764/45 and *SSW*-18/46, cited above) yielded Sul-r transformants which were selected on sulphadiazine-containing medium. Cultures on defined media later showed that the Sul-r transformants were Met⁻. These transformants were used in a second round of transformation in which a recipient population bearing the defective *met* determinant from *SSW*-18/46 was transformed to Met⁺ by treatment with DNA from the transformant bearing the non-identical *met* lesion of *SS*7764/45. The Met⁺ transformants selected on methionine-free defined medium were found subsequently to have regained susceptibility to sulphadiazine.[38] Because of the known antagonism between *p*-aminobenzoic acid and sulphonamides and the reports that higher concentrations of *p*-aminobenzoic acid were produced by Sul-r than by Sul-s gonococci,[39] the levels of this metabolic product were examined. No significant differences were found in the amounts of *p*-aminobenzoic acid produced by the Met⁻ and Met⁺ transformants.[38]

The genetic findings support the hypothesis that sulphonamide treatment of gonorrhoea was responsible for selecting Met⁻ mutants as well as sulphonamide-resistant mutants. Another effect of the drug was to eliminate many gonococci which were highly susceptible to sulphadiazine. Resistance of ⩽1 μg/ml characterised 40% of the gonococci listed in table III. In contrast, none of the 97 *N gonorrhoeae* isolates in 1975 were so susceptible,[7] although similar percentages of the two groups of gonococci showed resistance of ⩾35 μg/ml (11% and 9% respectively).

The advent of penicillin led to a reduction of the prevalence of sulphonamide-resistant gonococci from a peak of 85% in 1949-1950 to 5% of strains examined 10 years later.[24] Also, the decline in prevalence of Met⁻ gonococci testifies to a loss of the competitive advantage which methionine auxotrophy must have provided in the presence of a sulphonamide. The percentages of Met⁻ strains among North American isolates in the 1970s are reported to be 2·5% (15 of 600 isolates) in Rochester, New York[40]; 2·4% (6 of 251)[6] and 1% (1 of 97)[7] in Milwaukee, Wisconsin; 1·8% (2 of 114) in Seattle, Washington[8]; and 7·8% (19 of 243) in Hamilton, Ontario, Canada.[13] Three of 166 (1·8%) isolates in Stockholm, Sweden,[11] were Met⁻ as was one of 341 (0·3%) isolates in Strasbourg, France.[41] It will be of interest to monitor the prevalence of Met⁻ gonococci in communities where sulphamethoxazole-trimethoprim treatment is common.

Sulphonamides inhibit the biosynthesis of folic acid by their action on dihydropteroic acid synthetase, the enzyme responsible for incorporating *p*-aminobenzoic acid. Tetrahydrofolic acid coenzymes perform essential transfers of one-carbon units required for biosynthesis of various compounds, including methionine. Methionine biosynthesis in *Escherichia coli* is very sensitive to inhibition by sulphanilamide.[42] Recently, methionine (as a specific methionyl-tRNA) has been found to play a key role in the initiation of polypeptide synthesis.[43] The development of gonococcal resistance to sulphonamides involves at least two independent mechanisms. (a) Mutations leading to structural alterations of dihydropteroic acid synthetase selectively reduce its affinity for sulphanilamide compared with *p*-aminobenzoic acid.[44] (b) Mutations affecting one enzyme required in methionine biosynthesis secondarily enhance gonococcal resistance to sulphonamides; as yet, there is only circumstantial evidence for this mechanism and it has not been recognised in other genera. In enteric bacteria, the methylation of homocysteine to yield methionine may involve three enzymes specific to the methionine pathway, which are encoded by *met*F, *met*E, and *met*H.[45] A mutation affecting a gene homologous to one of these three genes is probably responsible for the Met$^-$ Sul-s phenotype of strains *G*-2 and *G*-4, whereas a mutation of a different *met* gene produced the phenotype of *G*-22 and other Met$^-$ Sul-r *N gonorrhoeae* strains. The observation that some Met$^-$ gonococci are Sul-s indicates that the requirement for an exogenous source of this critical end-product of folate metabolism does not of itself generate resistance to sulphonamides.

Part of this investigation was supported by Public Health Service Research grant 2 RO1 AI-02353 from the National Institute of Allergy and Infectious Diseases. The technical assistance of Patricia Zefran and Dorothy Casimere is gratefully acknowledged.

References

1. Reyn A, Korner B, Bentzon MW. Effects of penicillin, streptomycin, and tetracycline on *N gonorrhoeae* isolated in 1944 and in 1957. *Br J Vener Dis* 1958;**34**:227-39.
2. Willcox RR. A survey of problems in the antibiotic treatment of gonorrhoea with special reference to South-east Asia. *Br J Vener Dis* 1970;**46**:217-42.
3. Catlin BW. Evolution of *Neisseria gonorrhoeae*—1975. In: Schlessinger D, ed. *Microbiology—1976*. Washington DC: American Society for Microbiology, 1976;453-66.
4. World Health Organisation. *Neisseria gonorrhoeae* and gonococcal infections. *Technical Report Series* No 616. Geneva: World Health Organisation, 1978.
5. Catlin BW. Nutritional profiles of *Neisseria gonorrhoeae, Neisseria meningitidis,* and *Neisseria lactamica* in chemically defined media and the use of growth requirements for gonococcal typing. *J Infect Dis* 1973;**128**:178-94.
6. Carifo K, Catlin BW. *Neisseria gonorrhoeae* auxotyping: differentiation of clinical isolates based on growth responses on chemically defined media. *Appl Microbiol* 1973;**26**:223-30.
7. Exner AC, Shinners EN, Pace PJ, Catlin BW. Auxotypes and antibacterial resistance of gonococci with differing susceptibilities to vancomycin. *Br J Vener Dis* 1982;**58**:166-75.
8. Knapp JS, Holmes KK. Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. *J Infect Dis* 1975;**132**:204-8.
9. Morello JA, Lerner SA, Bohnhoff M. Characteristics of atypical *Neisseria gonorrhoeae* from disseminated and localized infections. *Infect Immun* 1976;**13**:1510-6.
10. Eisenstein BI, Lee TJ, Sparling PF. Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated gonococcal infection. *Infect Immun* 1977;**15**:834-41.
11. Moberg I. Auxotyping of gonococcal isolates. In: Danielsson D, Juhlin L, Mårdh P-A, eds. *Genital Infections and their Complications.* Stockholm: Almqvist & Wiksell International, 1975;271-3.
12. Knapp JS, Thornsberry C, Schoolnik GA, Wiesner PJ, Holmes KK, Co-operative Study Group. Phenotypic and epidemiologic correlates of auxotype in *Neisseria gonorrhoeae. J Infect Dis* 1978;**138**:160-5.
13. Hendry AT, Stewart IO. Auxanographic grouping and typing of *Neisseria gonorrhoeae. Can J Microbiol* 1979;**25**:512-21.
14. Thompson SE, Reynolds G, Short HB, Thornsberry C, Biddle JW, *et al.* Auxotypes and antibiotic susceptibility patterns of *Neisseria gonorrhoeae* from disseminated and local infections. *Sex Transm Dis* 1978;**5**:127-31.
15. Branham SE, Mitchell RH, Brainin W. Gonococcic meningitis. *JAMA* 1938;**110**:1804-6.
16. Catlin BW. *Neisseria meningitidis* (meningococcus). In: Lennette EH, Spaulding EH, Truant JP, eds. *Manual of Clinical Microbiology.* 2nd ed. Washington DC: American Society for Microbiology, 1974;116-23.
17. Catlin BW. Characteristics and auxotyping of *Neisseria gonorrhoeae.* In: Bergan T, Norris JR, eds. *Methods in Microbiology,* vol 10. London: Academic Press, 1978;343-80.
18. Catlin BW. Transfer of the organism named *Neisseria catarrhalis* to *Branhamella* gen nov. *Int J Syst Bacteriol* 1970;**20**:155-9.
19. Reyn A. Family I *Neisseriaceae.* In: Buchanan RE, Gibbons NE, eds. *Bergey's Manual of Determinative Bacteriology.* 8th ed. Baltimore: The Williams and Wilkins Co, 1974;427-32.
20. Umbarger HE. Amino acid biosynthesis and its regulation. *Ann Rev Biochem* 1978;**47**:533-606.
21. Catlin BW, Nash EH. Arginine biosynthesis in gonococci isolated from patients. In: Brooks GF, Gotschlich EC, Holmes KK, Sawyer WD, Young FE, eds. *Immunobiology of Neisseria gonorrhoeae.* Washington DC: American Society for Microbiology, 1978;1-8.
22. Shinners EN, Catlin BW. Arginine biosynthesis in *Neisseria gonorrhoeae:* enzymes catalyzing the formation of ornithine and citrulline. *J Bacteriol* 1978;**136**:131-5.
23. Thayer JD, Martin JE jun. Improved medium selective for cultivation of *N gonorrhoeae* and *N meningitidis. Public Health Rep* 1966;**81**:559-62.
24. Reyn A. Sensitivity of *N gonorrhoeae* to antibiotics. *Br J Vener Dis* 1961;**37**:145-57.
25. Lankford CE, Snell EE. Glutamine as a growth factor for certain strains of *Neisseria gonorrhoeae. J Bacteriol* 1943;**45**:410-1.
26. Lankford CE, Skaggs PK. Cocarboxylase as a growth factor for certain strains of *Neisseria gonorrhoeae. Arch Biochem* 1946;**9**:265-83.
27. Anonymous. Penicillin. *Br J Vener Dis* 1944;**20**:131-2.
28. Fleming A. Penicillin in venereal diseases. *Br J Vener Dis* 1944;**20**:133-6.
29. Harkness AH. Drug resistance in gonorrhoea with special reference to aetiology and treatment. *Br J Vener Dis* 1944;**20**:2-16.
30. Perdrup A. Gonorrhoea in Denmark, a survey of incidence over 82 years. *Br J Vener Dis* 1961;**37**:115-9.
31. Harrison LW. Anti-venereal measures in Denmark and Sweden. *Br J Vener Dis* 1939;**15**:1-17.
32. Magian AC. *The Practitioner's Manual of Venereal Diseases.* St Louis: CV Mosby Company, 1919.
33. Buttle GAH. The chemotherapy of sulphonamides. *Br J Vener Dis* 1939;**15**:83-99.
34. Demerec M, Hanson J. Mutagenic action of manganous chloride. *Cold Spring Harbor Symposia on Quantitative Biology* 1951;**16**:215-28.
35. Dees JE, Colston JAC. The use of sulfanilamide in gonococcic infections. *JAMA* 1937;**108**:1855-8.
36. Carpenter CM, Ackerman H, Winchester ME, Whittle J. Correlation of in vitro sulfonamide resistance of the gonococcus with results of sulfonamide therapy. *Am J Public Health* 1944;**34**:250-4.
37. Abrahamson RE. The effect of a respiratory sulfadiazine prophylactic program on the incidence of sulfonamide-resistant gonorrhea. *Am J Syph Gonorrhea Vener Dis* 1945;**29**:641-4.
38. Catlin BW. Genetic studies of sulfadiazine-resistant and methionine-requiring *Neisseria* isolated from clinical material. *J Bacteriol* 1967;**94**:719-33.

39. Landy M, Gerstung RB. *p*-Aminobenzoic acid synthesis by *Neisseria gonorrhoeae* in relation to clinical and cultural sulfonamide resistance. *J Immunol* 1945;**51**:269-77.
40. Young FE, Ploscowe V, Short H. The application of DNA-mediated transformation to elucidation of the pathobiology of *Neisseria gonorrhoeae*. In: Portolés A, López R, Espinosa M, eds. *Modern Trends in Bacterial Transformation and Transfection*. Amsterdam: North Holland Publishing Company, 1977:307-20.
41. Le Faou A, Guy I, Riou J-Y. Auxotypes et sensibilité à six antibiotiques des souches de *Neisseria gonorrhoeae* isolées à Strasbourg en 1977-1978. *Ann Dermatol Venereol* 1979;**106**: 267-72.
42. Winkler KC, de Haan PG. On the action of sulfanilamide. XII A set of noncompetitive sulfanilamide antagonists for *Escherichia coli*. *Arch Biochem* 1948;**18**:97-107.
43. Lodish HF. Translational control of protein synthesis. *Ann Rev Biochem* 1976;**45**:39-72.
44. Ho RI, Corman L, Morse SA, Artenstein MS. Alterations in dihydropteroate synthetase in cell-free extracts of sulfanilamide-resistant *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1974;**5**:388-92.
45. Whitehouse JM, Smith DA. Methionine and vitamin B_{12} repression and precursor induction in the regulation of homocysteine methylation in *Salmonella typhimurium*. *Molec Gen Genet* 1973;**120**:341-53.

Br J Vener Dis 1982;**58:**166-75

Auxotypes and antibacterial resistance of gonococci with differing susceptibilities to vancomycin

A CHARLES EXNER,* ELIZABETH NASH SHINNERS,* PAUL J PACE,† AND B WESLEY CATLIN*

*From the *Department of Microbiology, Medical College of Wisconsin, and the †Bureau of Laboratories, Milwaukee Health Department, Milwaukee, Wisconsin, USA*

SUMMARY The responses to vancomycin and 11 other antibacterial drugs and the nutritional requirements of gonococci recovered from two selective media were determined. Single urogenital specimens from 508 patients attending a social hygiene clinic in 1975 yielded 97 strains of *Neisseria gonorrhoeae;* 95 were recovered on VCNT (a modification of Thayer-Martin medium), always inoculated first, and 69 on LC medium containing lincomycin (4 μg/ml) and colistin (5 μg/ml). The two drugs at these concentrations in LC medium were not inhibitory for isolates from either medium. Unexpectedly, three isolates on VCNT were susceptible to vancomycin at the concentration (3 μg/ml) in VCNT medium; these three were typically sensitive to penicillins but were hypersusceptible to erythromycin (inhibited by ≤0·05 μg/ml) and rifampin (≤0·02 μg/ml). Resistance to streptomycin (≥500 μg/ml) (22% of the strains) was correlated with increased resistance to penicillins, erythromycin, and rifampin in most instances. All streptomycin-resistant gonococci required proline, or arginine, or none of the test compounds. Strains requiring arginine, hypoxanthine, and uracil were uniformly sensitive to antibiotics but not hypersusceptible. In contrast, six strains of *N gonorrhoeae* isolated in Denmark required arginine (not satisfied by ornithine), hypoxanthine, and uracil and were hypersusceptible to vancomycin (inhibited by 0·5 μg/ml), erythromycin, and rifampin. DNA-mediated transformation showed that all three hypersusceptibilities of one Danish strain were introduced together into a wild-type gonococcus, suggesting that a mutation of an *env* (envelope) locus might be responsible for the atypical permeability.

Introduction

Neisseria gonorrhoeae is an evolving species.[1] Strains isolated in the 1970s from different patients showed antigenic and nutritional differences and broad ranges of susceptibilities to various antibacterial drugs. Gonococci were uniquely susceptible to penicillin during the pre-penicillin era. Before 1945 the difference between the concentrations of penicillin required to inhibit the most susceptible and the least susceptible strains was no more than 10-fold.[2-4] A wider range of susceptibility occurred in gonococci isolated in 1949.[5] Resistance to penicillin increased worldwide during the 1950s and 1960s and necessitated progressively larger doses of penicillin for the treatment of gonorrhoea.[6-11] Penicillin resistance was often correlated with increased resistance to various other antibiotics.[10-14] While in some areas the trend towards increased resistance continued, in others the prevalence of gonococci resistant to one or more drugs, such as penicillin,[14 15] streptomycin,[16] and tetracycline,[17] notably decreased.

Furthermore, some gonococci have shown unusual degrees of susceptibility to various antibacterial compounds[18] including vancomycin.[8 15 19-27] On vancomycin-containing selective media, such as that of Thayer and Martin,[28] gonococci which are highly susceptible to vancomycin are inhibited. Nevertheless, the ability of marginally susceptible strains to form colonies on the primary culture medium is dependent on inoculum size[15 25-27] and on the physiological state of the bacteria.[24] When the diagnosis is not established patients with asymptomatic gonorrhoea may remain untreated, risking the development of complications. Moreover, a Danish

Address for reprints: Professor B Wesley Catlin, Department of Microbiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226, USA

Accepted for publication 23 December 1981

study[15] showed that continued transmission of vancomycin-hypersusceptible gonococci led to an increased percentage of such strains isolated in 1971 compared with 1969.

Gonococcal strains isolated in different communities not only show differences in their drug responses but also in their auxotypes. In Stockholm[29] and in Des Moines and Seattle, USA,[30] more than half of the *N gonorrhoeae* isolates had growth requirements for arginine (Arg), hypoxanthine (Hyx), and uracil (Ura), and possibly other compounds. Nevertheless, Arg-Hyx-Ura strains represented between only 6% and 25% of the isolates in nine other cities in the USA,[30-32] in Hamilton, Canada,[33] and Strasbourg, France.[34] Arg-Hyx-Ura strains are remarkably susceptible to penicillin and some other drugs.[27 30 33-35] This finding, together with the correlation between high susceptibility to penicillin and to vancomycin,[15 27] indicates that the vancomycin susceptibility and nutritional requirements of gonococci should be monitored in various communities.

In the present study 508 urogenital specimens, obtained in sequence from clinic patients, were cultured on two different selective media. All *N gonorrhoeae* isolates were examined for their nutritional requirements and levels of resistance to 12 antibacterial drugs with various mechanisms of action. In addition, tests were performed on six gonococcal strains found to be hypersusceptible to vancomycin when isolated in Copenhagen.[15] We used similar media and methods in this and the accompanying study[4] of gonococci recovered from patients in the pre-penicillin era. Accordingly, detailed comparisons of strains of *N gonorrhoeae* isolated 30 to 40 years apart were possible.

Patients and methods

SELECTIVE MEDIA

VCNT, a modification of Thayer-Martin medium,[28] contained vancomycin (3 μg/ml), colistin (7·5 μg/ml), nystatin (12·5 units/ml), and trimethoprim (5 μg/ml); this medium was supplied by Gibco Diagnostics, Madison, Wisconsin. LC medium contained lincomycin hydrochloride (Upjohn Co) and colistin (sodium colistimethate, Warner-Chilcott Laboratories) added to GCMBS in concentrations of 4 μg/ml and 5 μg/ml respectively. GCMBS was composed of GC medium base (Difco) with the White-Kellogg defined supplement.[35]

CULTURE TECHNIQUE

Cervical or urethral specimens were obtained in the summer of 1975 from 508 patients attending the Milwaukee Health Department Social Hygiene Clinic. Each specimen, collected with a cotton swab, was rolled in a "Z" pattern on the surfaces of plates of VCNT medium (always inoculated first) and LC medium. The media were cross-streaked with a bacteriological loop and promptly incubated at 35°C in an atmosphere of air plus CO_2 generated by a candle flame burning to extinction. After incubation for up to 48 hours the sets of primary cultures were processed independently, the VCNT cultures by health department bacteriologists and the LC cultures by two postgraduate students.

IDENTIFICATION OF GONOCOCCI

One or more isolates from 89 cervical specimens and from eight urethral specimens were confirmed as *N gonorrhoeae* by typical cellular and colonial morphology, positive oxidase reaction, production of acid from dextrose but not from maltose or sucrose, inability to hydrolyse *o*-nitrophenyl-β-D-galactopyranoside, and by a growth requirement for cysteine (or cystine). These gonococci were preserved at −60°C and designated by numbers and the prefix *SV* (when isolated from VCNT medium) or *SL* (from LC medium). The 97 *SV* and *SL* strains are the same 97 anogenital isolates given in the table in a previous report[36] for comparison with oropharyngeal isolates.

AUXOTYPING

The auxotypes of all *SV* and *SL* isolates were determined by their growth responses on standard *Neisseria* defined agar (NEDA) medium, which contained soluble starch 0·1% (w/v). The methods for preparing the media and the standardised gonococcal suspensions have been described.[35-37] All media were inoculated with between 10^3 and 10^4 colony-forming units delivered by the Steers replica-plating apparatus and incubated at 36°C in an atmosphere of air plus 8% CO_2.[37] The pattern of nutritional requirements, determined by the presence or absence of appreciable macroscopic growth on the set of NEDA media, defines the auxotype and is given as an abbreviation of each required compound.[37] Gonococci classified in the Zero auxotype do not require any of the compounds used for differentiating auxotypes. The arginine requirement of two strains was satisfied by arginine and citrulline but not by ornithine; this characteristic is designated Arg°.

SUSCEPTIBILITY TO ANTIBACTERIAL DRUGS

The gonococcal responses to 12 antibacterial drugs added to media in various concentrations were determined according to the methods described earlier.[35] In addition to *N gonorrhoeae* strain P-1T-1, we used the WHO reference strains, *N gonorrhoeae* III, V, and VII (supplied by A Reyn, Statens Seruminstitut, Copenhagen). Eight drugs were those listed previously[35]; the additional ones were lincomycin and colistin (specified above), vancomycin hydrochloride (Eli Lilly and Co), and nalidixic acid (generously supplied by William A Goss, Sterling-Winthrop Research Institute).

Assays of the benzylpenicillin resistance of all *SV* and *SL* isolates were performed by the Division of Laboratories of the Milwaukee Health Department using the standard haemoglobin-containing medium and the Steers multi-point inoculating apparatus.[35] Nevertheless, the gonococcal responses are given as degrees of resistance rather than as minimum inhibitory concentrations (MIC)—that is, as the highest concentration of penicillin or other drug that permitted growth of part of the population (at least five colonies); this was normally one concentration step lower than the MIC. Resistance expresses a definite characteristic of the population which relates to the possibility for continued growth and response to the selective effects of the drug.

GCMBS medium was used for determining the benzylpenicillin resistance of the strains listed in table III. The responses to the other 11 antibacterial drugs were also examined in GCMBS, although the determinations of resistance might be variously affected by the presence of certain biosynthetic intermediates (such as *p*-aminobenzoic acid), by the acid produced from dextrose (0·4%), and by the starch (0·1%) which binds some compounds.

Hypersusceptibility to vancomycin was introduced into a wild-type gonococcal strain by deoxyribonucleate (DNA)-mediated transformation.[38] DNA was extracted from the vancomycin-hypersusceptible strain *SS*158936/69, which had previously received a spectinomycin resistance marker by transformation. DNA treatment of the recipient gonococci[38] yielded vancomycin-hypersusceptible transformants which were isolated indirectly by selecting for co-transformants resistant to spectinomycin (25 μg/ml). The progeny of isolated transformants were screened for altered drug responses and nutritional requirements.

TABLE I *Auxotypes of 97 gonococcal isolates from 508 specimens from consecutive patients*

*Auxotype**	*No of strains*	*Auxotype**	*No of strains*
Zero	23	Pro-Hyx	1
Pro	29	Arg-Hyx-Ura	12
Arg	19	Arg-Hyx-Ura-Thp	2
Pro-Arg	3	Arg$^{\circ}$-Hyx-Ura	1
Hyx	2	Arg$^{\circ}$-Hyx-Ura-Leu	1
Thp	1	Arg-Hyx-Ura-Leu	1
His	1	Arg-Hyx-Ura-Met	1

*Zero auxotype strains do not require any of the compounds used for differentiating auxotypes. Other designations are given as abbreviations of each compound required: Pro, proline; Arg, arginine; Hyx, hypoxanthine; Thp, thiamine pyrophosphate; His, histidine; Ura, uracil; Leu, leucine; and Met, methionine; Arg$^{\circ}$ specifies a requirement for arginine that is not satisfied by ornithine.

Results

Strains of *N gonorrhoeae*, isolated on one or both selective media, were recovered from 97 (19·1%) of the urogenital specimens. Fourteen auxotypes were represented (table I). Auxotypes Zero, Pro, and Arg accounted for 73·2% of the 97 isolates. Of the remaining isolates, 18·6% were characterised by requirements that included arginine, hypoxanthine, and uracil. Similar percentages (76·9% and 17·5%, respectively) were found in the study of 251 *N gonorrhoeae* isolates from patients attending the same clinic in 1972.[31]

PRIMARY CULTURES ON VCNT AND LC MEDIA

The paired cultures from 508 single specimens yielded *N gonorrhoeae* in 95 VCNT cultures but in only 69 LC cultures. Two strains were recovered from LC medium only. The two corresponding VCNT cultures showed oxidase-positive colonies of Gram-negative diplococci which, however, failed to grow when subcultured. These two LC strains, representatives of the Zero and Pro-Arg auxotypes, were resistant to vancomycin at 15 and 25 μg/ml respectively.

VCNT cultures from 28 specimens yielded gonococci which were not recovered from LC medium. Four of these failures represented cultures overgrown by yeasts. The remaining 24 could not be explained by an unusual susceptibility to lincomycin, since the corresponding VCNT isolates were resistant to lincomycin at concentrations $\geqslant$10 μg/ml. Eight (33%) of these 24 strains exhibited nutritional requirements that included arginine, hypoxanthine, and uracil. The recovery percentages may have been affected by differences between the growth-promoting characteristics of the haemoglobin-containing basal medium used for VCNT and the GCMBS medium used for LC cultures. Also, a greater amount of contaminating growth has been found on media in which lincomycin was substituted for vancomycin[23]; contaminant colonies tend to obscure the small colonies typical of Arg-Hyx-Ura gonococci. Furthermore, specimens from some women with asymptomatic gonorrhoea may have contained too few gonococci to permit recovery from the LC medium which, according to the protocol, was always inoculated after the VCNT medium.

Auxotyping and susceptibility tests were performed on the paired VCNT and LC isolates from 63 specimens. The gonococci from a given specimen showed the same nutritional requirements. In most instances the values representing the drug responses of paired cultures were either identical or differed by only one concentration step. Wider deviations were found only for three drugs. Resistance to benzyl-

TABLE II *Drug responses of 76 streptomycin-susceptible (S) and 21 streptomycin-resistant (R) gonococcal strains arranged according to auxotype*

	No of strains according to auxotype:								
	Zero		*Pro*		*Arg*		*Pro-Arg*	*Others†*	
*Resistance (μg/ml)**	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*S*	*Total*
Vancomycin									
1·0	1	0	0	2	0	0	0	0	3
3·0	1	0	0	0	0	0	0	0	1
5·0	3	0	2	0	2	0	1	14	22
10	3	2	3	2	0	3	0	8	21
15	5	1	4	2	3	6	1	0	22
20	5	0	11	2	2	1	0	1	22
25	1	0	1	0	2	0	1	0	5
30	1	0	0	0	0	0	0	0	1
Lincomycin									
5	0	0	0	0	0	1	0	0	1
10	10	0	1	4	0	0	0	4	19
20	9	1	17	1	4	6	3	19	60
30	1	2	2	2	3	2	0	0	12
40	0	0	1	0	2	1	0	0	4
50	0	0	0	1	0	0	0	0	1
Tetracycline									
0·05	1	0	0	0	0	0	0	0	1
0·1	15	0	11	0	9	0	0	23	58
0·2	4	0	10	5	0	10	2	0	31
0·5	0	3	0	2	0	0	1	0	6
1·0	0	0	0	1	0	0	0	0	1
Chloramphenicol									
0·1	2	0	0	0	0	0	1	0	3
0·2	14	0	20	3	5	1	2	23	68
0·5	0	0	0	2	4	5	0	0	11
1·0	4	0	1	1	0	3	0	0	9
1·5	0	2	0	1	0	1	0	0	4
2·0	0	1	0	0	0	0	0	0	1
4·0	0	0	0	1	0	0	0	0	1
Nalidixic acid									
0·2	10	0	10	1	1	2	1	3	28
0·4	9	1	10	5	8	8	2	17	60
0·6	1	0	1	1	0	0	0	3	6
0·8	0	1	0	1	0	0	0	0	2
1·0	0	1	0	0	0	0	0	0	1
Sulphadiazine									
2	2	2	4	1	5	3	1	1	19
5	5	0	6	3	3	1	0	8	26
10	4	0	5	2	1	2	1	13	28
20	4	0	3	2	0	4	1	1	15
35	1	0	0	0	0	0	0	0	1
50	3	0	3	0	0	0	0	0	6
>100	1	1	0	0	0	0	0	0	2

*Resistance expressed as the highest concentration of antibacterial drug permitting growth
†Remaining 10 auxotypes listed in table I
S = susceptible; R = resistant

penicillin was 0·05 and 0·2 U/ml for one pair of Arg isolates and 0·1 and 0·3 U/ml for a pair of Zero auxotype isolates. Vancomycin resistance differed for three pairs of Pro isolates, being 10 and 30 μg/ml, 20 and 30 μg/ml, and 20 and 40 μg/ml. Sulphadiazine resistance was recorded as 2 and 10 μg/ml for one pair and 20 and 50 μg/ml for another pair of isolates.

Although other studies have shown that a patient may harbour two distinct strains of *N gonorrhoeae,*[32 35] only one possible instance was evident from the findings on the VCNT and LC cultures. An endocervical specimen (S260) yielded two isolates, both of which were characterised as Pro and resistant to benzylpenicillin at 0·1 U/ml. Nevertheless, these isolates differed consistently in repeated tests of resistance to streptomycin (5 and >500 μg/ml), chloramphenicol (1·0 and 0·2 μg/ml), rifampin (0·02 and 0·1 μg/ml), and vancomycin (25 and 10 μg/ml). Only the resistances of the streptomycin-susceptible strain are given in table II and figs 1 and 2.

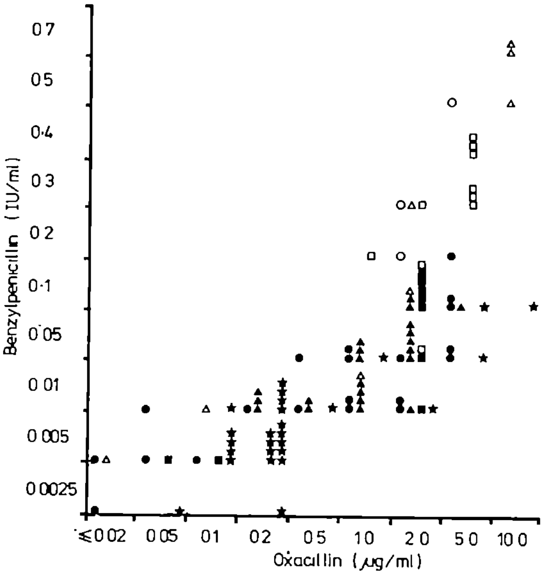


FIG 1 *Correlation between benzylpenicillin resistance and oxacillin resistance of 97 gonococcal strains subdivided according to auxotype and streptomycin response. Zero auxotype (● ○), Pro (▲△), Arg (■ □), all other auxotypes (★, 26 strains); strains sensitive to streptomycin (● ▲ ■ ★) or resistant to streptomycin (○ △ □). (See "patients and methods" for differences between assays of resistance to the two penicillins.)*

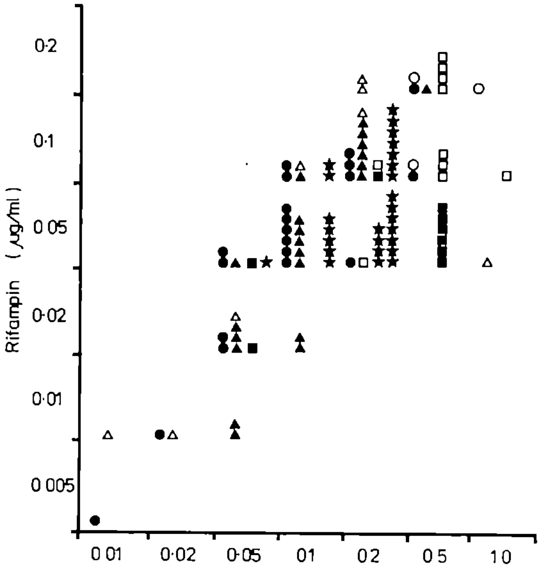


FIG 2 *Correlation between rifampin resistance and erythromycin resistance of 97 gonococcal strains subdivided according to auxotype and streptomycin response. Zero auxotype (● ○), Pro (▲△), Arg (■ □), all other auxotypes (★, 26 strains); strains sensitive to streptomycin (● ▲ ■ ★) or resistant to streptomycin (○ △ □).*

AUXOTYPES AND DRUG RESPONSES OF *SV* AND *SL* ISOLATES

The gonococcal responses to 11 of the 12 antibacterial drugs are summarised (table II, figs 1 and 2) for the two *SL* isolates not recovered on VCNT medium and for the 95 *SV* isolates. These were chosen for the sake of uniformity and because the related pairs of isolates did not differ significantly in most cases.

Vancomycin

Gonococci isolated from three specimens plated on VCNT medium unexpectedly failed to grow when assayed on GCMBS containing vancomycin at 3 µg/ml, the concentration in VCNT medium. In repeated tests strains *SV*6, *SV*12, and *SV*77 were resistant to vancomycin at 1·0 µg/ml but susceptible at 3·0 µg/ml (tables II and III). *SV*75 also failed to grow on vancomycin at 3·0 µg/ml in some tests, but since in others it gave sparse growth *SV*75 was given as resistant to 3·0 µg/ml. The remaining 93 strains, whether isolated on LC or on VCNT medium, were resistant to vancomycin at concentrations ≥5 µg/ml.

Lincomycin

The 71 members of the Zero, Pro, and Arg auxotypes showed a 10-fold range of resistance to lincomycin. The *SV* strains 6, 12, 75, and 77 were among the more susceptible with resistance limited to 10 µg/ml. The finding that all strains examined here and in the accompanying study[4] were resistant to at least 5 µg lincomycin/ml suggests the potential value of lincomycin as an alternative to vancomycin in selective culture media. Other investigators[11 23] have found, however, that some isolates are susceptible to lincomycin at ≤4 µg/ml.

Colistin

GCMBS medium containing colistin at 25 µg/ml supported growth of all 97 strains, and 85 strains were resistant to 75 µg/ml, the highest concentration tested (data not given).

Streptomycin

The gonococci isolated before 1945 were streptomycin-susceptible[3 4] and in 1957 only 2% of Danish strains were highly resistant.[6] Nevertheless, many studies of strains isolated after 1965 have shown that 20% or more were highly resistant to streptomycin and these often had increased resistance to penicillin and some other antibiotics.[10-14] Since streptomycin is not now the drug of choice for treatment of most diseases in the United States, we were interested in the prevalence and characteristics of resistant strains.

TABLE III *Characteristics of gonococci showing different levels of resistance to vancomycin*

*Laboratory No**	*Auxotype*	*Highest concentration of drug permitting growth (μg/ml)*								
		VAN	*PEN*	*OXA*	*RIF*	*ERY*	*CHL*	*STR*	*TET*	*SLD*
*SS*35503/68	A°HU†	0·2	0·01	0·1	0·01	0·05	0·2	5	0·1	10
*SS*156182/69	A°HU†	0·2	0·01	0·05	0·01	0·02	0·2	2	0·1	5
*SS*158296/69	A°HU†	0·2	0·01	0·05	0·01	0·02	0·2	2	0·1	1
*SS*158936/69	A°HU†	0·2	0·01	0·1	0·01	0·02	0·2	2	0·1	2
*SS*127462/69	A°HU†	0·2	0·01	0·05	0·01	0·02	0·2	2	0·1	5
*SS*54841/71	A°HU†	0·2	0·01	0·1	0·01	0·02	0·2	5	0·1	10
*SS*218767/72	A°HU†	5·0	0·01	0·2	0·05	0·2	0·2	2	0·1	2
*CHC*746	A°HU†	10·0	0·01	0·1	0·05	0·1	0·2	5	0·1	20
*SV*503	A°HU†	5·0	0·01	0·1	0·05	0·1	0·2	5	0·1	10
*SV*6	Zero	1·0	0·05	0·05	0·005	0·01	0·1	5	0·05	20
*SV*75	Zero	3·0	0·01	0·01	0·01	0·02	0·2	2	0·1	50
*SV*12	Pro	1·0	0·01	0·01	0·01	0·02	0·5	>500	0·2	10
*SV*77	Pro	1·0	0·05	0·1	0·01	0·01	0·5	>500	0·2	2
WHO III	Zero	10·0	0·05	0·1	0·005	0·02	0·2	5	0·05	20
*SS*41835/43	Zero	3·0	0·01	0·2	0·05	0·1	0·2	5	0·05	0·5
*SS*41835 transformant		0·2	0·01	0·1	0·01	0·02	0·2	NT	0·05	NT

NT = not tested; VAN = vancomycin, PEN = penicillin, OXA = oxacillin, RIF = rifampin, ERY = erythromycin, CHL = chloramphenicol, STR = streptomycin, TET = tetracycline, SLD = sulphadiazine

**SS* strains were isolated in Denmark in the year indicated by the last two digits[15]; *CHC*746 was isolated in 1974 from a culture of blood from a patient in Chicago; *SV* strains selected from table II; WHO III is a penicillin assay reference strain isolated in 1955; *SS*41835 transformant was derived by treatment of strain *SS*41835/43 with DNA from *SS*158936/69

†Arg°-Hyx-Ura = requirements for arginine (not satisfied by ornithine), hypoxanthine, and uracil

Twenty-two strains were resistant to streptomycin at a concentration ≥500 μg/ml (Str-r). As already mentioned, however, the results include the susceptible isolate from specimen S260 but not the Str-r isolate. The 76 strains referred to collectively as streptomycin-susceptible (Str-s) in table II and elsewhere had resistances ranging from 2 μg/ml (four strains) to 15 μg/ml (one strain); 37 strains were resistant to 5 μg/ml and 34 to 10 μg/ml. The 18 strains with requirements that included arginine, hypoxanthine, and uracil were Str-s. In contrast, there were 10 Str-r strains among the 19 strains which required arginine but not uracil.

Benzylpenicillin and oxacillin

The gonococci were more susceptible to benzylpenicillin than to oxacillin, as others have found.[10, 12] The range of resistance to benzylpenicillin of all Arg-Hyx-Ura strains was from 0·0025 to 0·01 U/ml (0·0015 to 0·006 μg/ml), whereas their resistance to oxacillin ranged from 0·1 to 0·5 μg/ml (fig 1). These strains and eight others not in the Zero, Pro, or Arg auxotypes are indicated by stars in figs 1 and 2. Three with requirements that included hypoxanthine, but not uracil, were resistant to benzylpenicillin at concentrations of 0·01 U/ml (Hyx, two strains) and 0·0025 U/ml (Pro-Hyx) and to oxacillin at 0·2, 0·1, and 0·05 μg/ml. Representatives of Thp, Pro-Arg, and His auxotypes showed greater resistances.

The Zero, Pro, and Arg strains showed a wide range of resistance to the two penicillins. Most of the Str-r strains (fig 1) had increased resistance to both penicillins but two Str-r Pro strains (*SV*12 and *SV*77) had only low resistance. Gonococci that were Str-r and unusually susceptible to oxacillin were described earlier.[12] Of the 19 Arg strains, 17 were resistant to oxacillin at concentrations ≥1·0 μg/ml and two (both Str-s, fig 1) were much less resistant (≤0·1 μg/ml).

The penicillin resistance of some strains was examined on GCMBS medium and on the starch-free, haemoglobin-containing medium. The benzylpenicillin resistance of strains *SV*503, *SV*75, and *SV*12 on GCMBS (table III) was 0·01 U/ml compared with 0·005 U/ml for the same strains tested on the starch-free medium (fig 1). Similarly, the resistance of strains *SV*6 and *SV*77 was 0·05 U/ml on GCMBS and 0·01 U/ml on the starch-free medium.

Rifampin and erythromycin

A positive correlation was found between the activities of rifampin and erythromycin (fig 2) and some other drugs.[13] Thus, the *SV* strains 6, 75, 12, and 77 were highly susceptible to rifampin and erythromycin (fig 2, table III) and more susceptible to the penicillins than most Zero or Pro strains. Correlated high resistances characterised many Str-r strains[6 12 14]; strains *SV*407 (Zero) and *SV*300 (Pro) had the highest resistances to erythromycin (1·0 μg/ml) and their resistances to other drugs were: rifampin, 0·2 and 0·05 μg/ml; benzylpenicillin, 0·5 and 0·7 U/ml; oxacillin, 5 and 10 μg/ml; tetracycline, 0·5 and 1·0 μg/ml; chloramphenicol, 2·0 and 4·0 μg/ml; and nalidixic acid, 1·0 and 0·8 μg/ml respectively.

Of the 19 Arg strains 17 were resistant to erythromycin at a concentration of $\geqslant$0·2 μg/ml whereas 27 of the 29 Pro strains were resistant at $\leqslant$0·2 μg/ml (fig 2). A uniformity of response to erythromycin (resistance, 0·1 or 0·2 μg/ml) and to rifampin (resistance, 0·05 or 0·1 μg/ml) characterised the Arg-Hyx-Ura strains and all but one strain (Thp auxotype) of the others represented in fig 2 by stars. These strains ("others" in table II) were remarkable also for their homogeneous responses to tetracycline and chloramphenicol.

Sulphadiazine

Representatives of the Zero and Pro auxotypes were the most resistant to sulphadiazine. No correlation was apparent between the responses to streptomycin and sulphadiazine. For example, the Str-r strains *SV*407 and *SV*300 had sulphadiazine resistances of 5 and 10 μg/ml respectively. Increased resistance to sulphadiazine was related to a requirement for methionine in gonococcal isolates in the sulphonamide era.[4] In the present study, however, the one methionine-requiring strain was resistant only to 5 μg sulphadiazine/ml.

CHARACTERISTICS OF VANCOMYCIN-SUSCEPTIBLE GONOCOCCI

A group of 125 strains of *N gonorrhoeae* inhibited by vancomycin at a concentration of 2 μg/ml and susceptible to low concentrations of penicillin, streptomycin, and tetracycline were isolated from Danish patients.[15] Six of these strains, which were isolated over three years, were kindly provided by Alice Reyn. All six consistently failed to grow on GCMBS medium containing vancomycin at 0·5 μg/ml but were resistant to 0·2 μg/ml (table III). They all had the same pattern of requirements for arginine (not satisfied by ornithine), hypoxanthine, and uracil and hypersusceptibility to erythromycin and rifampin. The resistance of three other Arg°-Hyx-Ura strains and a Zero auxotype strain, *SS*41835/43, are included in table III for comparison. *SS*41835/43 presumably bears antibiotic response loci representative of "wild type" since it was isolated in 1943 during the pre-penicillin era.[4]

Sparling and his colleagues[18 39] identified pleiotropic chromosomal mutations (of *env*A and *env*B) that result in non-specific increased sensitivity to hydrophilic antibiotics, such as erythromycin (MIC $\leqslant$0·12 μg/ml), rifampin, and fusidic acid, and to dyes and detergents. The *env* mutations also produced complete phenotypic suppression of *mtr* (multiple resistance) and *pen*B (two loci encoding low-level resistances to multiple drugs) as well as decreased cross-linking of peptidoglycan in the cell wall. These genetic studies[18 39 40] did not, however, examine the possible influence of *env* mutations on vancomycin susceptibility. The responses of the WHO reference strain III (table III) show that hypersusceptibility to erythromycin and rifampin may be associated with a typical level of resistance to vancomycin.

To determine whether the mutant gene locus responsible for vancomycin hypersusceptibility also affected the responses to erythromycin and rifampin, we treated populations of the wild-type strain *SS*41835/43 with transforming DNA from the vancomycin-hypersusceptible strain *SS*158936/69. Four transformants derived in separate experiments were converted to vancomycin-hypersusceptibility and concurrently each became more susceptible to erythromycin and rifampin. Table III shows the characteristics of one transformant which, like the other three, was unchanged with respect to nutritional requirements and responses to benzylpenicillin, chloramphenicol, and tetracycline. Further genetic studies are needed to determine whether the mutant locus introduced into the transformant corresponds to *env*A or *env*B and also whether *env* mutations are responsible for the marginal vancomycin susceptibility of the *SV* strains 6, 12, and 77.

Discussion

N gonorrhoeae has been subjected to the selective effects of penicillins since the mid-1940s. Before then gonococci had developed certain nutritional requirements, but none of the pre-penicillin era isolates required uracil.[4] In contrast, many contemporary strains require uracil as a result of genetic defects of up to three pyrimidine biosynthetic enzymes (carbamyl phosphate synthetase, aspartate transcarbamylase, and orotate phosphoribosyltransferase) (Shinners and Catlin, unpublished data). Gonococci bearing these mutations also require arginine and, frequently, hypoxanthine. Although, inexplicably, the Arg-Hyx-Ura strains remain antibiotic-susceptible, many representatives of the Zero, Pro, and Arg auxotypes developed drug resistances (figs 1, 2, and table II).[30 32 35 41]

The variety of phenotypes is a reflection of the genetic versatility of *N gonorrhoeae*. Some chromosomal gene mutations alter ribosomal target sites resulting in high-level resistance to streptomycin or spectinomycin. The mutations of other genes produce only small effects individually but interact co-operatively to enhance resistances to penicillin, tetracycline, erythromycin, chloramphenicol, and some other drugs.[1 39 42] In the 1970s the progressive increase in such resistances appeared to level out or, in some regions, to reverse.[8 14-17 41] Thus, over several years the Greenland treatment regimen (5 megaunits benzylpenicillin intramuscularly preceded by oral probenecid) resulted in a pronounced increase in the proportion of penicillin-susceptible gonococci compared with moderately resistant strains.[43 44] Familiar explanations for this include possible differences in infectivity and the importation of susceptible strains from other communities. It is important, however, to recognise that the penicillin-susceptible gonococci isolated at present may differ genetically from the susceptible strains of the past.

Recently, Arg-Hyx-Ura strains have been responsible for many cases of asymptomatic gonorrhoea in men.[45] Requirements for compounds, such as hypoxanthine and uracil, that may be present in inadequate concentrations in healthy mucous membranes would limit gonococcal multiplication and so lessen the development of the purulent discharge seen in gonorrhoea caused by nutritionally independent strains. Anomalous findings of slightly higher penicillin failure rates in patients infected with highly susceptible gonococci compared with patients with strains of intermediate susceptibilities have been reported.[17] The persistence of such genetically susceptible gonococci in penicillin-treated patients may represent phenotypic resistance in cells whose growth is limited by unsatisfied nutritional requirements. Because penicillins act by inhibiting the cross-linking of peptidoglycan of the cell wall, non-multiplying gonococci will survive.[1 41] These two examples of the theoretical consequences of growth limitation may partially explain the increased prevalence of Arg-Hyx-Ura strains and, accordingly, the increased proportion of strains susceptible to streptomycin and other antibiotics.

This explanation for the decline of resistant strains is not applicable in all communities, however. In Milwaukee, 44 of 251 (17·5%) isolates of *N gonorrhoeae* examined in a previous study[31] required arginine, hypoxanthine, and uracil; of 169 strains (selected at random), 74 (44%) were resistant to streptomycin (Carifo and Catlin, unpublished data). In contrast, in this study of gonococci isolated three years later from patients attending the same clinic the percentage of streptomycin-resistant isolates had decreased to 22% although the proportion of Arg-Hyx-Ura strains remained constant (18·6%).

The potential effects of *env* mutations on the resistance of present-day isolates must be recognised. Mutations of *env* loci both increase the cellular permeability, and hence the susceptibility to various unrelated drugs, and also suppress the activities of mutant genes responsible for multiple low-level resistances.[39 40] A study in North Carolina showed that approximately 15% of *N gonorrhoeae* isolates possessed *env* mutations that cause hypersusceptibility to several compounds including erythromycin.[18] The WHO reference strain III, which was isolated in 1955,[46] and the *SV* strains 6, 75, 12, and 77 had resistances to erythromycin and rifampin of <0·02 and <0·01 μg/ml respectively (table III). These responses represent hypersusceptibilities, in as much as the concentrations are lower than those found for any of the pre-penicillin *N gonorrhoeae* isolates.[4] Strains *SV* 6, 12, and 77, but not WHO III, are marginally hypersusceptible to vancomycin as well. The six Arg°-Hyx-Ura Danish isolates[15] are hypersusceptible to erythromycin, rifampin, and vancomycin. The ability of transforming DNA from one of these Arg°-Hyx-Ura strains to introduce all three hypersusceptibilities into wild-type recipient populations of *SS*41835/43 (table III) suggests that the correlated hypersusceptibilities might be due to one mutation of an *env* locus.

We examined gonococcal responses to the two penicillins because of the possibility that an envelope alteration in a strain typically susceptible to benzylpenicillin might be detected by an unusual susceptibility to the bulky oxacillin molecule. *SV*75 and *SV*12 were notable for their relatively similar susceptibilities to benzylpenicillin (0·01 U/ml, equivalent to 0·006 μg/ml) and to oxacillin (0·01 μg/ml). In contrast, the six Arg°-Hyx-Ura strains with correlated hypersusceptibilities displayed substantially greater resistance to oxacillin than to benzylpenicillin. The variety of hypersusceptibility patterns given here suggests that increased susceptibilities as well as increased resistances to multiple drugs are consequences of the co-operative actions of multiple mutant gene loci.

The *SV* strains 6, 12, and 77 were recovered from VCNT medium although subsequent tests found that these three, together with two corresponding isolates from LC medium (*SL*12 and *SL*77), were repeatedly inhibited by vancomycin incorporated in GCMBS medium at a concentration of 3 μg/ml. This unexpected finding, also reported by others,[26] might be explained by differences between the basal media or the inoculum size. Nevertheless, we also considered that the activity of the vancomycin may have declined during refrigeration of the VCNT plates before their use in the clinic. Unfortunately, the expiration dates were not recorded because we did not foresee the possibility that gonococci having marginal resistance might form colonies on medium containing vancomycin with a reduced potency when used late in its shelf-life (established by the manufacturer as eight weeks).

Much attention has been devoted to problems concerning increased resistance to antibiotics, and numerous reports have documented increased susceptibilities.[19-27] The observation that the percentage of vancomycin-hypersusceptible isolates increased over a two-year period in Denmark[15] necessitates continuing studies of hypersusceptible gonococci in other countries. If two primary culture media containing antibacterial agents are to be used, one medium should incorporate drugs which differ from vancomycin in their modes of action.

This investigation was supported in part by Public Health Service research grant 2 R01 AI02353 from the National Institute of Allergy and Infectious Diseases and by the Communicable Disease Control and Vaccination Assistance Act—317, Program to Control Venereal Disease.

References

1. Catlin BW. Evolution of *Neisseria gonorrhoeae*—1975. In: Schlessinger D, ed. *Microbiology—1976*. Washington DC: American Society for Microbiology, 1976;453-66.
2. Lankford CE. The in-vitro tolerance of the gonococcus for penicillin. *Am J Syph Gonor Vener Dis* 1945;**29**:56-63.
3. Reyn A. Sensitivity of *N gonorrhoeae* to antibiotics. *Br J Vener Dis* 1961;**37**:145-57.
4. Catlin BW, Reyn A. *Neisseria gonorrhoeae* isolated from disseminated and localised infections in pre-penicillin era: auxotypes and antibacterial drug resistances. *Br J Vener Dis* 1982;**58**:158-65.
5. Gocke, TM, Wilcox C, Finland M. Antibiotic spectrum of the gonococcus. *Am J Syph Gonor Vener Dis* 1950;**34**:265-71.
6. Reyn A, Korner B, Bentzon MW. Effects of penicillin, streptomycin, and tetracycline on *N gonorrhoeae* isolated in 1944 and in 1957. *Br J Vener Dis* 1958;**34**:227-39.
7. WHO Expert Committee on Gonococcal Infections. First report. *WHO Tech Rep Ser No 262*. Geneva: World Health Organization, 1963:1-70.
8. Reyn A. Recent developments in the laboratory diagnosis of gonococcal infections. *Bull WHO* 1969;**40**:245-55.
9. Martin JE jun, Lester A, Price EV, Schmale JD. Comparative study of gonococcal susceptibility to penicillin in the United States, 1955-1969. *J Infect Dis* 1970;**122**:459-61.
10. Willcox RR. A survey of problems in the antibiotic treatment of gonorrhoea with special reference to South-east Asia. *Br J Vener Dis* 1970;**46**:217-42.
11. Phillips I, Rimmer D, Ridley M, Lynn R, Warren C. In-vitro activity of 12 antibacterial agents against *Neisseria gonorrhoeae*. *Lancet* 1970;i:263-5.
12. Reyn A, Bentzon MW. A study of the relationships between the sensitivities of *Neisseria gonorrhoeae* to sodium penicillin G, four semi-synthetic penicillins, spiramycin, and fusidic acid. *Br J Vener Dis* 1968;**44**:140-50.
13. Meheus A, Piot P, Pattyn S, Van Dyck E, Vanden Berghe D. Activity in vitro of 10 antimicrobial agents against *Neisseria gonorrhoeae*. *Br J Vener Dis* 1976;**52**:329-32.
14. Platt DJ. Prevalence of multiple antibiotic resistance in *Neisseria gonorrhoeae*. *Br J Vener Dis* 1976;**52**:384-6.
15. Reyn A, Bentzon MW. Comparison of a selective and a non-selective medium in the diagnosis of gonorrhoea to ascertain the sensitivity of *Neisseria gonorrhoeae* to vancomycin. *Br J Vener Dis* 1972;**48**:363-8.
16. Seth AD, Kolator B, Wilkinson AE. Sensitivity of *Neisseria gonorrhoeae* to antibiotics in London (1976-78). *Br J Vener Dis* 1979;**55**:325-8.
17. Jaffe HW, Biddle JW, Thornsberry C, *et al*. National gonorrhea therapy monitoring study. *N Engl J Med* 1976;**294**:5-9.
18. Eisenstein BI, Sparling PF. Mutations to increased antibiotic sensitivity in naturally-occurring gonococci. *Nature* 1978;**271**:242-4.
19. Cross RC, Hoger MB, Neibaur R, Pasternack B, Brady FJ. VCN-inhibited strains of *Neisseria gonorrhoeae*. *HSMHA Hlth Rep* 1971;**86**:990-2.
20. Phillips I, Humphrey D, Middleton A, Nicol CS. Diagnosis of gonorrhoea by culture on a selective medium containing vancomycin, colistin, nystatin and trimethoprim (VCNT). *Br J Vener Dis* 1972;**48**:287-92.
21. Brorson JE, Holmberg I, Nygren B, Seeberg S. Vancomycin-sensitive strains of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1973;**49**:452-3.
22. Ødegaard K, Solberg O, Lind J, Myhre G, Nyland B. Lincomycin in selective medium for the isolation of *Neisseria gonorrhoeae*. *Acta Pathol Microbiol Scand (B)* 1975;**83**:301-4.
23. Faur YC, Weisburd MH, Wilson ME. The selectivity of vancomycin and lincomycin in NYC medium for the recovery of *N gonorrhoeae* from clinical specimens. *Hlth Lab Sci* 1978;**15**:22-7.
24. Platt DJ, Gerken A. Inhibition of gonococci by a selective medium: disparity between isolates from sexual partners. *Br J Vener Dis* 1979;**55**:65.
25. Taylor E, Phillips I. Assessment of a selective medium for the isolation of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1979;**55**:183-5.
26. Windall JJ, Hall MM, Washington II JA, Douglass TJ, Weed LA. Inhibitory effects of vancomycin on *Neisseria gonorrhoeae* in Thayer-Martin medium. *J Infect Dis* 1980;**142**:775.
27. Mirrett S, Reller LB, Knapp JS. *Neisseria gonorrhoeae* strains inhibited by vancomycin in selective media and correlation with auxotype. *J Clin Microbiol* 1981;**14**:94-9.
28. Thayer JD, Martin JE jun. Improved medium selective for cultivation of *N gonorrhoeae* and *N meningitidis*. *Public Health Rep* 1966;**81**:559-62.
29. Moberg I. Auxotyping of gonococcal isolates. In: Danielsson D, Juhlin L, Mårdh P-A, eds. *Proceedings of the Symposium on Genital Infections and their Complications*. Stockholm: Almqvist and Wiksell International, 1975:271-3.
30. Knapp JS, Thornsberry C, Schoolnik GA, *et al*. Phenotypic and epidemiologic correlates of auxotype in *Neisseria gonorrhoeae*. *J Infect Dis* 1978;**138**:160-5.
31. Carifo K, Catlin BW. *Neisseria gonorrhoeae* auxotyping: differentiation of clinical isolates based on growth responses on chemically defined media. *Appl Microbiol* 1973;**26**:223-30.
32. Noble RC. Characterisation of *Neisseria gonorrhoeae* from women with simultaneous infections at two sites. *Br J Vener Dis* 1980;**56**:3-5.
33. Stewart IO, Hendry AT. Association between the auxogroup of *Neisseria gonorrhoeae* and the minimal inhibitory concentration of penicillin. *Sex Transm Dis* 1979;**6**:247-52.
34. Le Faou A, Guy I, Riou J-Y. Auxotypes et sensibilité à six antibiotiques des souches de *Neisseria gonorrhoeae* isolées à Strasbourg en 1977-1978. *Ann Dermatol Venereol* 1979;**106**:267-72.
35. Catlin BW, Pace PJ. Auxotypes and penicillin susceptibilities of *Neisseria gonorrhoeae* isolated from patients with gonorrhoea involving two or more sites. *Antimicrob Agents Chemother* 1977;**12**:147-56.
36. Catlin BW, Pace PJ. Nutritional requirements and penicillin susceptibilities of gonococci from pharyngeal and anogenital sites. *Br J Vener Dis* 1977;**53**:299-303.
37. Catlin BW. Characteristics and auxotyping of *Neisseria gonorrhoeae*. In: Bergan T, Norris JR, eds. *Methods in Microbiology*, vol 10. London: Academic Press, 1978:345-80.
38. Catlin BW. Genetic transformation of biosynthetically defective *Neisseria gonorrhoeae* clinical isolates. *J Bacteriol* 1974;**120**:203-9.

39. Sparling PF, Sox TE, Mohammed W, Guymon LF. Antibiotic resistance in the gonococcus: diverse mechanisms of coping with a hostile environment. In: Brooks GF, Gotschlich EC, Holmes KK, Sawyer WD, Young FE, eds. *Immunobiology of Neisseria gonorrhoeae*. Washington DC: American Society for Microbiology, 1978:44-52.
40. Guymon LF, Walstad DL, Sparling PF. Cell envelope alterations in antibiotic-sensitive and -resistant strains of *Neisseria gonorrhoeae*. *J Bacteriol* 1978;**136**:391-401.
41. Eisenstein BI, Lee TJ, Sparling PF. Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated gonococcal infection. *Infect Immun* 1977;**15**:834-41.
42. Warner PF, Zubrzycki LJ, Chila M. Polygenes and modifier genes for tetracycline and penicillin resistance in *Neisseria gonorrhoeae*. *J Gen Microbiol* 1980;**117**:103-10.
43. Olsen GA. Consumption of antibiotics in Greenland, 1964-1970. IV Changes in the sensitivity of *N gonorrhoeae* to antibiotics. *Br J Vener Dis* 1973;**49**:33-41.
44. Evans AJ, Morrison GD, Price DJ. Prolonged use of the Greenland method of treatment of gonorrhoea. *Br J Vener Dis* 1980;**56**:88-91.
45. Crawford G, Knapp JS, Hale J, Holmes KK. Asymptomatic gonorrhoea in men: caused by gonococci with unique nutritional requirements. *Science* 1977;**196**:1352-3.
46. Reyn A, Bentzon MW, Thayer JD, Wilkinson AE. Results of comparative experiments using different methods for determining the sensitivity of *Neisseria gonorrhoeae* to penicillin G. *Bull WHO* 1965;**32**:477-502.

Br J Vener Dis 1982;**58**:176-9

Single oral dose of cefaclor for the treatment of infections with penicillinase-producing strains of Neisseria gonorrhoeae

THELMA E TUPASI, OFELIA V CALUBIRAN, AND CLEOTILDE A TORRES
From the Infectious Disease Section, Department of Medicine, College of Medicine, University of the Philippines, Manila, Philippines

SUMMARY A single oral dose of 3 g cefaclor was effective in the treatment of uncomplicated gonococcal infection in women due to penicillinase-producing strains of *Neisseria gonorrhoeae* (PPNG). Cefaclor was equally active in vitro against both PPNG (MIC range, 0·01-1·0 μg/ml) and non-PPNG strains (MIC range, 0·005-2·0 μg/ml). As empirical treatment cefaclor was effective in 53 of 57 (93%) patients compared with ampicillin, to which only 37 of 53 (69·8%) patients responded. This difference was attributed to the 40% incidence of PPNG in the patients studied; as expected, such patients responded poorly to ampicillin.

Introduction

Cefaclor is an acid-stable semisynthetic cephalosporin which differs from its parent substance, cephalexin, in the substitution of a chloro group in the 3 position. Like cephalexin, cefaclor is absorbed orally but has the advantage of greater in-vitro activity against pathogens, particularly *Haemophilus influenzae* and *Neisseria gonorrhoeae* and their β-lactamase-positive strains.[1 2] Compared with other cephalosporins cefaclor was second only to cefuroxime in its activity against *N gonorrhoeae*.[2]

Preliminary studies of the treatment of gonococcal urethritis in men have shown cefaclor to be effective in doses ranging from 2-4 g with or without probenecid.[3] Its efficacy in infections with penicillinase-producing *N gonorrhoeae* (PPNG) has not yet been studied. The purpose of this study was to determine the efficacy of cefaclor in the treatment of uncomplicated gonococcal infections in women, particularly those due to PPNG. The results of empirical treatment of gonococcal infection with this drug were compared to those with the standard oral dose of ampicillin. Minimum inhibitory concentrations (MIC) of cefaclor, ampicillin, and penicillin against the isolates of *N gonorrhoeae* were measured and correlated with the response to treatment.

Address for reprints: Dr T E Tupasi, Department of Medicine, College of Medicine, University of the Philippines, Pedro Gil Street, Manila, Philippines

Accepted for publication 7 November 1981

Patients and methods

STUDY DESIGN

Prostitutes with uncomplicated gonococcal infection confirmed by culture on screening volunteered to join the study after the protocol had been explained. Written informed consent was obtained and the study was conducted in accordance with the guidelines for human experimentation of the Declaration of Helsinki and the Committee on Research, Ethics, and Development of the UP Health Sciences Center.

Pretreatment culture specimens were obtained from the cervix, urethra, rectum, and pharynx, after which patients were assigned to either treatment group by random allocation using a table of random numbers.

The patients were admitted to the clinic for three days after treatment to observe for possible side effects and, more importantly, to preclude reinfection before test-of-cure cultures were obtained on the second and third post-treatment days. Only patients with confirmed gonococcal infection based on a positive pretreatment culture result were evaluated. Treatment was considered to have failed if post-treatment culture results were still positive for *N gonorrhoeae.*

TREATMENT REGIMENS

Treatment consisted of a single oral dose of either 3 g cefaclor or 3·5 g ampicillin with 1 g probenecid. Patients who were randomly allocated to the ampicillin group and from whose pretreatment cultures PPNG were subsequently isolated were retreated with spectinomycin 2 g intramuscularly after post-treatment culture results were obtained and immediately before discharge.

STATISTICAL ANALYSIS

Fisher's exact test and the χ^2 test with Yates's correction were used.

BACTERIOLOGICAL TECHNIQUES

N gonorrhoeae was isolated and identified according to standard procedures.[4] Penicillinase production by the isolated strains of *N gonorrhoeae* was determined by the rapid iodometric test[5] and confirmed by the chromogenic cephalosporin method of O'Callaghan.[6] The MICs of cefaclor, ampicillin, and penicillin were determined by the agar dilution method using standard procedures.[7]

OTHER LABORATORY INVESTIGATIONS

Complete blood cell count, urine analysis, and serum alanine aminotransferase (ALT), blood urea nitrogen, and serum creatinine concentrations were determined before treatment and 24 hours later.

Results

STUDY POPULATION

A total of 122 patients were initially enrolled in the study; those in each treatment group were comparable for age, weight, and sexual exposure. Since penicillinase production of the infecting strain could not be determined from the pretreatment culture at the time of the patient's inclusion into the study, the patients were not grouped according to this criterion. Thus, using random allocation, it was solely by chance that more patients with PPNG strains were assigned to the cefaclor treatment group.

Twelve patients, including four given cefaclor and eight given ampicillin, were excluded from the study because of negative pretreatment culture results. Interestingly, in one such patient given cefaclor with a negative pretreatment culture result, post-treatment cultures from the cervix and rectum subsequently gave a heavy growth of PPNG. Despite the precautions taken against reinfection during the study, this could not be totally excluded, as the patient may have left the clinic without the knowledge of the clinic staff.

The ages of the remaining 110 patients ranged from 15 to 34 years with a mean of 20 years, and weights ranged from 36·3 kg to 61·3 kg with a mean of 45·5 kg. Thirty-eight of 110 (34·5%) patients were symptomatic; 44 patients were infected with PPNG and 66 with non-PPNG strains of *N gonorrhoeae*. Twenty-seven of the former and 30 of the latter were treated with cefaclor and the remainder received ampicillin.

TABLE I *Results of treatment in 122 patients with uncomplicated gonococcal infection*

Treatment outcome	No of patients harbouring strains of: PPNG	Non-PPNG	Total
Cefaclor (n = 61)*			
Response	27	26	53
Failure	0	4	4
Total			57
Ampicillin (n = 61)*			
Response	1	36	37
Failure	16	0	16
Total			53

*Of the two treatment groups, four and eight patients were excluded from the analysis as their pretreatment culture results were negative for *N gonorrhoeae*

PPNG = penicillinase-producing *N gonorrhoeae*; n = No of patients treated

TREATMENT

Fifty-three of the 57 (93%) patients treated with cefaclor responded to treatment compared with 37 of 53 (69·8%) patients treated with ampicillin. This difference was statistically significant ($\chi^2_1 = 8{\cdot}416$, $P<0{\cdot}01$). Of patients with PPNG infections, all 27 responded to cefaclor while 16 of 17 (94·1%) failed to respond to ampicillin (table I). The single patient who responded to ampicillin had only a few colonies of PPNG in her pretreatment culture from the urethra; after treatment no organisms were cultured from this site. In patients with non-PPNG infections the therapeutic response was significantly better with ampicillin ($P = 0{\cdot}038$ by Fisher's exact test) since no failure was noted with this drug compared with four failures out of 30 (13·3%) with cefaclor.

MINIMUM INHIBITORY CONCENTRATIONS

The correlation between the treatment response with cefaclor and the corresponding MICs of cefaclor for the infecting strains are shown in table II. Four treatment failures occurred among patients infected with

TABLE II *Minimum inhibitory concentrations of infecting N gonorrhoeae strain from patients treated with cefaclor in relation to treatment failures*

	No of patients harbouring strains of:			
	PPNG		Non-PPNG	
MIC (μg/ml)	Treated	Failed	Treated	Failed
≤0·025	1	0		
0·1	3	0	4	1
0·2			7	0
0·5	15	0	14	1
1·0	6	0	4	2
≥2·0			1	0
ND	2	0	0	0
Total	27	0	30	4

PPNG = penicillinase-producing *N gonorrhoeae*; ND = not done non-PPNG strains; of these, two had MICs of 1·0 μg/ml, one of 0·5 μg/ml, and one of 0·1 μg/ml. Two of 25 (8%) patients whose infecting strains had MICs of <0·5 μg/ml cefaclor failed to respond compared with two of five (40%) with MICs of >1·0 μg/ml cefaclor; this difference was not statistically significant (P=0·019 by Fisher's exact test). Sixteen patients who failed to respond to ampicillin had infecting strains of PPNG with MICs ranging from 4·0->64 μg/ml of ampicillin.

The MICs of cefaclor, ampicillin, and penicillin for the isolates are shown in table III. The MICs of the three antibiotics were not determined in a few of the isolates, which could not be retrieved from the stock cultures. The MIC range of cefaclor for PPNG strains was 0·01-1·0 μg/ml with 84·2% of isolates inhibited by 0·5 μg/ml and was similar to that for non-PPNG strains at 0·005-2·0 μg/ml with 87·1% of isolates inhibited by 0·5 μg/ml. Cefaclor was slightly more active in vitro than ampicillin and penicillin against non-PPNG strains; the ampicillin MIC range was 0·05-8·0 μg/ml with 79% of isolates inhibited by 0·5 μg/ml while the penicillin MIC range was 0·01-4·0 μg/ml with 75·4% of isolates inhibited by 0·5 μg/ml. The PPNG strains were uniformly resistant to ampicillin and penicillin with MIC ranges of 4·0->64 μg/ml and 16·0->64 μg/ml respectively.

SIDE EFFECTS

Three of 57 (5·3%) patients given cefaclor had side effects of dizziness (one) and dizziness, headache, and nausea (two). In contrast, only one of 53 (1·9%) patients given ampicillin complained of dizziness. Ten patients treated with cefaclor showed changes in blood analysis (eosinophilia in six, leucopenia in two, leucocytosis in one, and a mildly raised ALT concentration in one) compared with 14 treated with ampicillin (eosinophilia in seven, leucopenia in three, leucocytosis in two, and a mildly raised ALT concentration in two).

TABLE III *Cumulative percentage of isolates inhibited by increasing concentrations of cefaclor, ampicillin, and penicillin*

	% of isolates inhibited by:					
	Cefaclor		*Ampicillin*		*Penicillin*	
MIC (μg/ml)	*PPNG*	*Non-PPNG*	*PPNG*	*Non-PPNG*	*PPNG*	*Non-PPNG*
<0·005		1·6				
0·01	2·6	1·6				7·7
0·02	5·3	4·8				10·8
0·05	5·3	11·3		6·4		26·2
0·1	31·6	38·7		37·1		33·8
0·2	36·8	58·1		64·5		46·2
0·5	84·2	87·1		79·0		75·4
1·0	100	98·4		95·2		87·7
2·0		100		98·4		95·4
4·0			2·7	98·4		100
8·0			8·1	100		
16·0			21·6		8·1	
32·0			40·5		43·2	
>64·0			100		100	
No tested	38	62	37	62	37	65
No not tested*	6	4	7	4	7	1

*Isolates not retrieved from stock cultures for MIC determination
PPNG = penicillinase-producing *N gonorrhoeae*

Discussion

With the recently reported isolation of a spectinomycin-resistant strain of PPNG[8] an appraisal of the efficacy of the cephalosporins as an alternative treatment for gonococcal infection has become imperative. Despite earlier discouraging results of the treatment of gonorrhoea with older cephalosporins, such as cephaloridine, cefazolin, and cephalexin,[9-12] newer congeners (notably cefuroxime and cefamandole) and a cephamycin, cefoxitin, have shown excellent in-vitro activity against *N gonorrhoeae.*[13] Cefuroxime and cefoxitin have been highly effective in the treatment of gonococcal infections, including those due to PPNG.[4 14] In addition to these drugs, our study shows that cefaclor, an oral cephalosporin, has excellent in-vitro activity against *N gonorrhoeae* and was effective at a dose of 3 g in the treatment of uncomplicated gonococcal infections in women particularly those due to PPNG. Its oral route of administration is an important advantage over cefuroxime and cefoxitin, which may make it more acceptable to patients. As empirical treatment before the results of tests for penicillinase production of the infecting strain were known, cefaclor was more efficacious than ampicillin (93% responded to cefaclor compared with 69·8% to ampicillin). This significant difference ($\chi^2_1 = 8\cdot416$, P<0·01) is attributed to the 40% incidence of PPNG infections in the patients studied. As expected, such patients responded poorly to β-lactamase-sensitive drugs like ampicillin and penicillin.

The treatment failure rate among patients infected with non-PPNG strains was 13·3% with cefaclor compared with zero failure with ampicillin. In-vitro data suggest that cefaclor should be as effective as ampicillin, so that the significant difference (P=0·038 by Fisher's exact test) in the treatment results might be attributed to differences in the pharmacokinetics of the two drugs, most likely enhanced by the concomitant administration of probenecid with ampicillin. Pharmacokinetic studies indicate that while cefaclor is rapidly absorbed in the gastrointestinal tract, it is cleared more rapidly than cephalexin from the serum[15] and has a half-life of 0·76 hours after a 1-g dose with no detectable concentrations six hours later.[16] Conceivably, probenecid given with cefaclor in the treatment of gonococcal infection may ensure higher and prolonged concentrations of cefaclor. This potential benefit remains to be determined.

This project was supported by the National Research Council of the Philippines and the Eli Lilly Co.

References

1. Silver MS, Counts GW, *et al.* Comparison of in-vitro antibacterial activity of the three oral cephalosporins: cefaclor, cephalexin and cephaloridine. *Antimicrob Agents Chemother* 1977;**12**:591-6.
2. Hall WH, Schierl EA, *et al.* Comparative susceptibility of penicillinase-positive and negative *Neisseria gonorrhoeae* to 30 antibiotics. *Antimicrob Agents Chemother* 1979;**15**:562-7.
3. Spagna VA, Perkins RL, *et al.* Successful treatment with cefaclor of gonococcal urethritis in men. *Sex Transm Dis* 1979; 6:211-3.
4. Tupasi TE, Crisologo LB, *et al.* Single-dose therapy in 562 cases of uncomplicated gonorrhea in confined female cases. Proceedings of the 11th ICC and the 19th ICAAC American Society for Microbiology. *Current Chemotherapy and Infectious Diseases* 1980;**2**:1270-2.
5. Catlin BW. Iodometric detection of *Hemophilus influenzae* beta-lactamase: rapid presumptive test for ampicillin resistance. *Antimicrob Agents Chemother* 1975;7:265-70.
6. O'Callaghan CB, Kirby SM, Morris A, Shingler AH. Correlation between hydrolysis of the lactam bond of cephalosporin nucleus and expulsion of the 3 substituent. *J Bacteriol* 1972; **110**:988-91.
7. Biddle JW, Swenson JM, Thornsberry C. Disc agar diffusion antimicrobial susceptibility test with β-lactamase-producing *Neisseria gonorrhoeae. J Antibiot* 1978;**31**:352-7.
8. Anonymous. Spectinomycin-resistant penicillinase-producing *Neisseria gonorrhoeae. Morbidity and Mortality Weekly Report* 1981;**30**:221.
9. Oller LZ, Smith HG, Marshall MJ. Cephaloridine and cephalexin in venereological practice. *Postgrad Med J* 1970; **46** suppl:99-102.
10. Willcox RR, Woodcock KS. Cephalexin in the oral treatment of gonorrhoea by a double-dose method. *Postgrad Med J* 1970;**46** suppl:103-6.
11. Karney WW, Turck M, Holmes KK. Cefazolin in the treatment of gonorrhea. *J Infect Dis* 1973;**128** suppl:399-402.
12. Nelson M. Cefazolin in the treatment of uncomplicated gonorrhea in men. *J Infect Dis* 1973;**128** suppl:404-6.
13. Barry AL, Thornsberry C, Jones RN, Fuchs PC, Gavan TL, Gerlach EH. Cefuroxime, an in-vitro comparison with six other cephalosporins. *Proc R Soc Med* 1977;**70** suppl:63-71.
14. Berg SW, Kilpatrick ME, Harrison WO, McCutchan JA. Cefoxitin as a single-dose treatment for urethritis caused by penicillinase-producing *Neisseria gonorrhoeae. N Engl J Med* 1979;**301**:509-11.
15. Wise R, Andrews JM, Dean S, Welling PG, Kendall MJ. A pharmacological and in-vivo comparison of three oral cephalosporins. *J Antimicrob Chemother* 1979;**5**:601-7.
16. Hodges GR, Liu C, Hinthorn R, Harms JL, Dworzack DI. Pharmacological evaluation of cefaclor in volunteers. *Antimicrob Agents Chemother* 1978;**14**:454-6.

Br J Vener Dis 1982;**58:**180-1

Treatment of uncomplicated gonorrhoea in women with talampicillin in a single oral 1·5 g dose*

K C MOHANTY AND R B ROY

From the Department of Genitourinary Medicine, Royal Victoria Hospital, Bournemouth, Dorset

SUMMARY Four hundred and sixty women with uncomplicated gonorrhoea were treated with a single oral dose of 1·5 g talampicillin. The success rate among those who attended for at least one follow-up examination after treatment was 99·14%. The antibiotic was well tolerated and no side effects were reported. Talampicillin given under supervision has proved to be the most effective and safest treatment in this area. A smaller dosage of talampicillin than ampicillin is required, the drug is better tolerated by the patient, and the time of nursing staff is saved.

Introduction

Talampicillin, the phthalidyl ester of ampicillin, is well absorbed from the gastrointestinal tract. During absorption it is hydrolysed by non-specific esterases to release ampicillin, thus increasing the bioavailability of ampicillin to three times the values achieved with an equivalent dose of ampicillin.[1] Ampicillin has now become recognised worldwide as a standard single-dose oral treatment.[2] Moreover, the penicillin group of antibiotics exerts its influence on the logarithmic phase of gonococci, which is very short (30-40 minutes), and the number of ampicillin-sensitive strains of gonococci have increased favourably during the late 1970s.[3] Talampicillin has been used with great success in the treatment of gonorrhoea in men.[2 4 5] Since no similar study has so far been reported in women, we decided to undertake a clinical trial with talampicillin in a single oral dose of 1·5 g for the treatment of uncomplicated gonorrhoea in women.

Patients and methods

CLINICAL DATA

Four hundred and ninety-six women with uncomplicated gonorrhoea diagnosed by microscopy of Gram-stained smears and confirmed by culture were included in the study. Of these, 477 (96·16%) were British, 389 (78·4%) were single, and 221 (44·55%) were aged 20-24 years. Ninety-two (18·54%) women had vaginal discharge, vaginal irritation, and dysuria; 44 (8·87%) had had gonorrhoea in the past; 347 (69·9%) attended the clinic with contact slips; and 476 (95·97%) had acquired the disease locally.

DIAGNOSIS

All patients had a genital examination performed at their initial visit and subsequently during follow up. In 421 (84·87%) patients both microscopy of cervical and urethral smears and culture results for *Neisseria gonorrhoeae* were positive, and in 75 (15·1%) culture results alone were positive. Patients whose results for Gram-stained smears were positive and whose culture results were negative were excluded from the study. Microscopy of wet-mount preparations and culture of vaginal specimens were performed to exclude *Trichomonas vaginalis* and *Candida albicans.* Serological tests for syphilis were carried out on all patients at their initial visit and repeated 4-6 weeks later and, when possible, after three months.

TREATMENT AND FOLLOW UP

All 496 patients were treated with talampicillin 1·5 g in a single dose. The importance of follow-up examination was explained to the patients at their initial visit and reattendance was requested seven, 14, 28, and 42 days after the first day of treatment. These intervals were not always rigidly observed. Patients were advised to avoid sexual intercourse until the results of three consecutive sets of tests were negative and to abstain from alcohol for 48 hours; these restrictions were probably not always adhered to.

Results

Of the 496 women treated with 1·5 g talampicillin 460 attended at least one follow-up examination after

---

*Paper read at the Spring Meeting of the MSSVD, Bournemouth, May 1980

Address for reprints: Dr R B Roy, Department of Genitourinary Medicine, Royal Victoria Hospital, Gloucester Road, Bournemouth BH7 6JF

Accepted for publication 19 January 1982

TABLE *Comparison of results of single-dose oral treatment of uncomplicated gonorrhoea in women*

Treatment	*No of cases followed*	*Failure rate* *No*	*Failure rate* *%*	*Study*	*Year*
Ampicillin 2 g + probenecid 1 g	393	9	2·3	Brø-Jorgenson and Jensen[7]	1972
Amoxicillin 1 g + probenecid 1 g	37	5	21·7	Thin *et al*[8]	1977
Rifampicin 300 mg	42	6	8·6	Panduro[9]	1971
Co-trimoxazole*	275	17	6·1	Rahim[10]	1975
Vibramycin 300 mg	219	10	5·7	Enfors and Eriksson[11]	1975
Pivampicillin 1·4 g + probenecid 1 g	79	2	2·5	Forström[12]	1974
Ampicillin 1 g + probenecid 1 g	546	9	1·55	Roy (unpublished data)	1977
Talampicillin 1·5 g	460	4	0·86	Present study	1980

*Trimethoprim 640 mg + sulphamethoxazole 32 g

treatment. Only four (0·86%) treatment failures occurred during the second week after treatment, and 20 (4·3%) recurrences in patients who admitted to further sexual exposure with casual or untreated partners were cured with the same dosage of talampicillin. These 20 patients were probably reinfections. No side effects were reported by any of the patients.

Discussion

Ampicillin with or without probenecid has been proved to be successful in the treatment of uncomplicated gonorrhoea in men.[6] The present study showed that talampicillin gave a very high cure rate (99·14%) in uncomplicated gonorrhoea in women probably because almost all the patients acquired the infection locally and all the strains were sensitive to talampicillin (MIC = 0·03 μg/ml). None of the isolates from the four treatment failures produced β-lactamase, and all four were resistant to penicillin (MIC = 0·25 μg/ml). These strains were acquired outside the locality (Bangkok, Middlesborough, Liverpool, and London). All four patients responded to 4 g spectinomycin intramuscularly.

The results with talampicillin alone in the treatment of uncomplicated gonorrhoea in women thus proved to be superior to those obtained with single doses of other antibiotics (table). The high cure rate may not be achieved in other countries or indeed in the large conurbations of Britain, since the prevalence of highly insensitive strains of *N gonorrhoeae* varies in different areas. With variations in the sensitivity pattern of gonococci in the future, this single-dose regimen may possibly become less successful. Its performance must therefore be monitored regularly by determining the clinical failure rate and the sensitivity of the isolates.

We are grateful to our colleagues and staff for their co-operation, particularly to Mr D Boyd, SRN, for his help, and to Mrs E Weatherstone, for her secretarial help.

References

1. Jones KH. Bioavailability of talampicillin. *Br Med J* 1977;ii:232-3.
2. Price JD, Fluker JL, Giles AJH. Oral talampicillin in the treatment of gonorrhoea. *Br J Vener Dis* 1977;**53**:113-4.
3. Seth AD, Kolator B, Wilkinson AE. Sensitivity of *Neisseria gonorrhoeae* to antibiotics in London. *Br J Vener Dis* 1979;**55**:325-8.
4. Willcox RR. Single oral dose of 1·5 g talampicillin in the treatment of gonorrhoea. *Br J Vener Dis* 1976;**52**:184-6.
5. Al-Egaily, Dunlop EMC, Rodin P, Seth AD. Talampicillin and probenecid compared with ampicillin and probenecid for the treatment of gonococcal urethritis in men. *Br J Vener Dis* 1978;**54**:242-6.
6. Roy RB, Laird SM. Procaine penicillin alone compared with ampicillin plus probenecid in the treatment of gonorrhoea in the male. *Br J Vener Dis* 1974;**50**:117-9.
7. Brø-Jorgensen A, Jensen T. Single-dose oral treatment of gonorrhoea in men and women using ampicillin alone and combined with probenecid. *Br J Vener Dis* 1971;**47**:443-7.
8. Thin RN, Symonds MAE, Shaw EJ, Wong J, Hopper PK, Slocombe B. A double-blind trial of amoxycillin in the treatment of gonorrhoea. *Br J Vener Dis* 1977;**53**:118-20.
9. Panduro J. Treatment of acute gonorrhoea with single oral dose of rifampicin. *Br J Vener Dis* 1971;**47**:440-2.
10. Rahim G. Single-dose treatment of gonorrhoea with co-trimoxazole. *Br J Vener Dis* 1975;**51**:179-82.
11. Enfors W, Eriksson G. Comparison of oral ampicillin and doxycycline in the treatment of uncomplicated gonorrhoea. *Br J Vener Dis* 1975;**51**:99-103.
12. Forström L. Pivampicillin HCL-probenecid combination in the treatment of uncomplicated gonorrhoea. *Br J Vener Dis* 1974;**50**:61-3.

Br J Vener Dis 1982;**58**:182-3

Therapeutic abortion and Chlamydia trachomatis infection

ERIK QVIGSTAD,* KJELL SKAUG,† FRIDTJOF JERVE,* INGER S S VIK,†
AND JAN C ULSTRUP†

*From the *Department of Obstetrics and Gynaecology, and the †Microbiological Laboratory, Ullevaal Hospital, Oslo, Norway*

SUMMARY *Chlamydia trachomatis* was isolated from the cervix of 30 of 218 (13·8%) women admitted for legal termination of pregnancy. During the first two weeks after the abortion seven of the 30 (23·3%) patients developed pelvic inflammatory disease. Four of these had serological evidence of recent active chlamydial infection. Thus, routine examination of patients for genital chlamydial infection before termination of pregnancy is recommended.

Introduction

The presence of *Chlamydia trachomatis* in the cervix is a frequent causal factor in the epidemiology of pelvic inflammatory disease (PID).[1-4] One episode of PID may result in tubal dysfunction; tubal patency is rapidly impaired by recurrent infections and may later cause infertility.[5] PID due to *C trachomatis* may also occur without subjective symptoms.[3]

PID is a major complication after therapeutic abortion; the readmission rate in our hospital is about 4%, pelvic infections and retained products being the main causes.[6]

The aim of this study was to record the incidence of *C trachomatis* infections among patients admitted for legal abortion and to follow those harbouring chlamydia, particularly those in whom it caused postoperative infections.

Patients and methods

STUDY POPULATION

Two hundred and eighteen women admitted consecutively for termination of pregnancy in the first trimester in 1980 were included in the study. Abortion was carried out by dilatation and vacuum aspiration in all patients.

The diagnosis of PID was made on the clinical grounds of pelvic pain, adnexal masses, increased erythrocyte sedimentation rate, and fever. The patients who developed acute salpingitis were treated with doxycycline.

Patients who harboured *C trachomatis* were recalled for follow up about three months after the abortion.

ISOLATION OF *C TRACHOMATIS* AND *N GONORRHOEAE*

Before surgery specimens for culture of *C trachomatis* and *N gonorrhoeae* were obtained from the cervix and cultured within 24 hours. The chlamydial specimens were collected with cotton-tipped swabs and transported in a sucrose phosphate buffer (2SP). *C trachomatis* was cultured in cycloheximide-treated McCoy cells.[7]

Specimens for culture of *N gonorrhoeae* were collected with charcoal-treated cotton-tipped swabs and transported in a modified Stuart medium. *N gonorrhoeae* was cultured on a modified Thayer-Martin medium and identified by sugar fermentation tests.

SEROLOGY

Before abortion and at follow-up examination serum samples were examined for chlamydial IgG antibodies by an enzyme-linked immunosorbent assay (ELISA).[8] The ELISA was performed on flat-bottomed microtitre plates coated with a suspension of partially purified cell-cultured *C trachomatis* subtype LGV-2 particles. Antibody titres were expressed as the highest reciprocal serum dilution showing at least twice the mean absorbance value of a group of negative sera. A titre >1/8 was defined as a positive result. A four-fold or more rise in titre was regarded as evidence of actual chlamydial infection.

Results

CULTURE

Of the 218 patients, *C trachomatis* was isolated from the cervix in 30 (13·8%), *N gonorrhoeae* in six (2·8%), and both *C trachomatis* and *N gonorrhoeae* in two.

Address for reprints: Dr E Qvigstad, Department of Obstetrics and Gynaecology, Ullevaal Hospital, Oslo 1, Norway

Accepted for publication 19 November 1981

Seven of the 30 (23·3%) patients harbouring *C trachomatis* developed PID. All the infections occurred in the first two weeks after the abortion. None of the patients with cervical gonorrhoea developed salpingitis.

FOLLOW UP

Twenty-one of the 30 chlamydia-positive patients attended for follow up three months after the termination of pregnancy (table). Of the seven patients with pelvic infection, six attended (patients 1-6); four of these had an appreciable rise in chlamydial IgG antibody titre while two had raised but unchanged titres. A further four patients (Nos 7-10) had a four-fold or more rise in titre but no clinical evidence of infection.

TABLE *Chlamydial IgG antibody titres in paired serum samples from 21 patients harbouring C trachomatis by an enzyme-linked immunosorbent assay before abortion and at follow up*

	Serum IgG antibody titres	
Patient No	*First sample**	*Second sample†*
1	32	128
2	8	32
3	8	32
4	128	512
5	32	32
6	64	64
7	<8	2048
8	8	512
9	32	512
10	32	256
11	16	16
12	8	8
13	32	32
14	<8	<8
15	<8	<8
16	32	32
17	128	128
18	32	32
19	128	128
20	64	64
21	<8	<8

* Sample collected before abortion
† Sample collected at follow up

Discussion

The existence of pathogenic micro-organisms in the cervix before therapeutic abortion is of importance, particularly because of the risk of ascending infection. The incidence of chlamydial infection of the cervix in women undergoing termination of pregnancy is between 6% and 13%.[9] In the present study *C trachomatis* was isolated in 13·8% and *N gonorrhoeae* in 2·8% of patients.

Most chlamydial infections are confined to the epithelial cells of the mucous membranes of the genital tract. The antigenic stimulus is minimal and the humoral antibody response poor. The use of a sensitive serodiagnostic test is, therefore, important. In the present study an ELISA, which has the same sensitivity and specificity as the single-antigen immunofluorescence test,[4, 8] was used to detect chlamydial IgG antibodies. Of the 21 patients harbouring *C trachomatis* who were followed up, 17 (81%) had detectable chlamydial IgG antibodies; of these, eight had an appreciable rise in antibody titre. All the six patients who were followed up and developed salpingitis after termination of pregnancy had chlamydial antibodies. These results agree with those obtained by the micro-immunofluorescence test.[10, 11]

The study indicates that patients harbouring *C trachomatis* in the cervix at termination of pregnancy are at high risk of developing postoperative infections and that *C trachomatis* is a major aetiological agent in salpingitis occurring after legal abortion. Routine screening for *C trachomatis* in the cervix before surgery is thus essential. Although only the chlamydia-positive patients were followed up in this study, all patients will be examined thoroughly before and after therapeutic abortion in future studies.

References

1. Mårdh P-A, Helin I, Bobeck S, Laurin J, Nilsson T. Colonisation of pregnant and puerperal women and neonates with *Chlamydia trachomatis. Br J Vener Dis* 1980;**56**:96-100.
2. Mårdh P-A, Ripa T, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977;**296**:1377-9.
3. Punnonen R, Terho P, Nikkanen V, Meurman O. Chlamydial serology in infertile women by immunofluorescence. *Fertil Steril* 1979;**31**:656-9.
4. Vik ISS, Skaug K, Qvigstad E, Ulstrup JC, Jerve F. Isolation and serological diagnosis of *Chlamydia trachomatis* in acute salpingitis. *Tidsskr Nor Lægeforen* (in press).
5. Weström L. Effect of acute pelvic inflammatory disease on fertility. *Am J Obstet Gynecol* 1975;**121**:707-13.
6. Jerve F, Fylling P. Therapeutic abortion. *Acta Obstet Gynecol Scand* 1978;**57**:237-40.
7. Ripa T, Mårdh P-A. New simplified culture technique for *Chlamydia trachomatis*. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology 1977;323-7.
8. Skaug K, Vik ISS, Qvigstad E, Ulstrup JC, Jerve F. Chlamydial serum IgG antibodies in patients with acute salpingitis measured by an enzyme-linked immunosorbent assay. *Acta Pathol Microbiol Scand (C)* (in press).
9. Schachter J. Chlamydial infections. *N Engl J Med* 1978; **298**:540-9.
10. Treharne JD, Dines RJ, Darougar S. Serological responses to chlamydial ocular and genital infections in the United Kingdom and Middle East. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections*. Washington DC; American Society for Microbiology, 1977:249-58.
11. Wang S-P, Grayston JT, Kuo C-C, Alexander ER, Holmes KK. Serodiagnosis of *Chlamydia trachomatis* infection with the micro-immunofluorescence test. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections*. Washington DC: American Society for Microbiology, 1977: 237-48.

Br J Vener Dis 1982;**58**:184-7

Some factors affecting the incubation period and duration of symptoms of urethritis in men

C B S SCHOFIELD

From Ward 34, Newcastle General Hospital, Newcastle upon Tyne

SUMMARY In a retrospective study of clinic records containing accurate information on the dates of infection and onset of symptoms, the mean (± SEM) incubation period of gonorrhoea in men was 6·2 ± 3·8 days and the mean duration of symptoms 3·1 ± 2·3 days. For non-specific urethritis the mean (± SEM) figures were 7·7 ± 4·1 and 4·0 ± 3·4 days respectively; both were significantly longer than for gonorrhoea.

Patients with a first episode of urethritis had longer than average incubation periods and duration of symptoms. Past experience of urethritis shortened the duration of symptoms; this was more significant in gonorrhoea than in non-specific urethritis. "Anxious" men who had attended previously of their own accord when no abnormality had been found had the shortest times. The most notable factor in determining the interval between infection and attendance, however, was whether or not the sexual contact was known. Infections by known contacts were associated with prolonged duration of symptoms which negated the benefit of past experience and, to some extent, of anxiety.

Thus, patients should be made more generally aware that known contacts may be a source of infection, especially after a break in a relationship, and that they should return to the clinic as soon as symptoms develop. Furthermore, doctors should examine carefully even the most persistently "neurotic" patients, because they may eventually become infected.

Introduction

The incubation period of urethral gonorrhoea in men is variously quoted as being from one to 10 days (but mostly five days or less) by King *et al*,[1] from two to five days by Catterall[2] and Morton[3]—who notes that some authorities mention an extreme of 2-3 weeks[3]—from three to five days by Robertson *et al*,[4] and an average of 7·9 days.[5]

The incubation period of non-specific urethritis (NSU) is even less precise: from five to 28 days,[2] from one to three weeks,[1] from one to four weeks,[5] from a few days to a few weeks,[4] and, for half the patients, over 10 days.[3]

The duration of symptoms before patients attend for treatment is ignored by all except one author, who quotes a mean period of 4·5 days for gonorrhoea[5] but does not mention that for NSU. It is unfortunate that little interest is paid to this period during which the patients are symptomatic and infectious, as it could be shortened by effective health education.

The aim of this study was to determine the mean incubation periods and duration of symptoms of gonorrhoea and NSU in men and to evaluate the effects of inexperience in those with a first episode of urethritis (group A), of experience of urethritis in the past (group B), of "anxiety" (as implied by those who had attended previously of their own accord but without any detectable abnormality) (group C), and of being infected by known or unknown contacts.

Patients and methods

STUDY CRITERIA

To determine the mean incubation period and duration of symptoms in patients with gonorrhoea and NSU the clinic records for 1978 were studied retrospectively. The type of attendance (groups A-C) and whether or not the sexual contact was known to the patient were noted.

The criteria for inclusion in the study were:

(a) A record of the exact dates of infection and of the onset of symptoms.

Address for reprints: Dr C B S Schofield, Ward 34, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE

Accepted for publication 1 December 1981

TABLE I *Gonorrhoea and non-specific urethritis in men. Mean incubation periods and duration of symptoms by initial or previous attendance (days ± SEM)*

	No (%) of men with:		*Mean (± SEM) incubation periods (days):*		*Mean (± SEM) duration of symptoms (days):*		*Total periods*	
Attendance groups	*Gonorrhoea*	*NSU*	*Gonorrhoea**	*NSU†*	*Gonorrhoea‡*	*NSU§*	*Gonorrhoea*	*NSU*
A: initial urethritis	178 (73·6)	151 (57·2)	6·6 ± 3·8	8·2 ± 3·8	3·4 ± 2·4	4·4 ± 3·6	10·0	12·6
B: repeated urethritis	46 (19·0)	96 (36·4)	5·4 ± 3·3	7·4 ± 4·4	2·5 ± 1·4	3·4 ± 3·1	7·9	10·8
C: no previous abnormality	18 (7·4)	17 (6·4)	4·4 ± 2·7	5·0 ± 3·3	1·6 ± 1·2	3·3 ± 3·1	6·0	8·3
Totals	242 (100·0)	264 (100·0)	6·2 ± 3·8	7·7 ± 4·1	3·1 ± 2·3	4·0 ± 3·4	9·3	11·7

Statistical differences:
* A *vs* B, not significant; B *vs* C, not significant; A *vs* C, significant (P=0·002).
† A *vs* B, not significant; A *vs* C, significant (P<0·01); B *vs* C, significant (P<0·01)
‡ A *vs* B *vs* C, significant (P<0·01)
§ A *vs* B, significant; (P<0·02)
SEM = standard error of mean

(b) A past history of:

- (i) No previous episode of urethritis but possible attendance with other conditions.
- (ii) Previous attendance with urethritis of any sort.
- (iii) No abnormality found on previous attendance for routine examination.

(c) The source of infection noted as known or unknown contact in the clinic records or the health adviser's contact cards.

STATISTICAL ANALYSIS

Statistical significances of differences were determined by the χ^2 test and by Student's t test (with Bessel's correction for small numbers) for differences of means.

Results

The criteria for inclusion in the study were fulfilled by 242 men with gonorrhoea, 51·6% of the 1978 total, and by 264 with NSU (excluding those with post-gonococcal urethritis), 27·4% of the total. Ninety per cent of those with gonorrhoea and 95% of those with NSU were born in the North-east of England.

The mean (±SEM) incubation period for patients with gonorrhoea was 6·2 (±3·8) days and for those with NSU 7·7 (±4·1) days (table I). For those with gonorrhoea the only significant difference was between those with a first episode of urethritis (group A) and those who were "anxious" (group C) (table I). For patients with NSU the differences between groups A and C and between groups B and C were significant (table I).

The mean (±SEM) duration of symptoms for patients with gonorrhoea was 3·1 (±2·3) days and that for those with NSU 4·0 (±3·4) days. For gonorrhoea the differences between all three groups were significant (P<0·01 for all three). For NSU the only difference of possible significance was that between groups A and B (P = 0·02) (table I).

KNOWN AND UNKNOWN CONTACTS

No significant difference in the mean incubation periods of gonorrhoea was found between those infected by known or unknown contacts (table II), both groups consisting of similar proportions of the three types of attendance (0·5>P>0·3). For NSU (also with similar proportions of the three types of attendance) (P>0·5), however, the difference was significant (P = 0·001) and was due solely to the difference among those attending with a first episode of urethritis (P<0·001). For both gonorrhoea and NSU the mean durations of symptoms of those infected by known contacts were significantly longer

TABLE II *Gonorrhoea and non-specific urethritis in men. Mean (± SEM) incubation periods and durations of symptoms by whether contact known or unknown (in days)*

	No (%) of patients with:		*Mean (± SEM) incubation periods (days):*		*Mean (± SEM) duration of symptoms (days):*		*Total periods*	
Contact	*Gonorrhoea*	*NSU*	*Gonorrhoea*	*NSU*	*Gonorrhoea*	*NSU*	*Gonorrhoea*	*NSU*
Known	78 (32·2)	106 (40·2)	6·9 ± 4·2	9·0 ± 4·2	4·9 ± 2·7	6·0 ± 4·3	11·8	15·0
Unknown	164 (67·8)	158 (59·8)	5·9 ± 3·6	6·8 ± 3·7	2·2 ± 1·1	2·6 ± 1·7	8·1	9·4
Totals	242 (100·0)	264 (100·0)	6·2 ± 3·8	7·7 ± 4·1	3·1 ± 2·3	4·0 ± 3·4	9·3	11·7

SEM = standard error of mean

(P<0·001 for both) than of those infected by unknown contacts.

For further analysis the three types of attendance were combined with knowledge or otherwise of the sexual contacts (table III). For both gonorrhoea and NSU the two longest mean incubation periods and durations of symptoms were associated with known contacts. In contrast the two shortest incubation periods were associated with group C and the shortest duration of symptoms with unknown contacts of group C. Thus, no one factor influenced the incubation periods, but the durations of symptoms were predominantly determined by the type of contact; the three longest were associated with known contacts and the three shortest with unknown contacts, whereas within each of the three types of attendance the duration decreased from group A to group B to group C.

Discussion

The mean incubation period (6·2 days) and duration of symptoms (3·1 days) for patients with gonorrhoea were shorter than reported previously[5] (7·9 and 4·5 days respectively). This may be due to the more rigid selection of patients in the present study. Both the mean incubation periods (7·7 days) and the duration of symptoms (4·0 days) for NSU were significantly longer than those for gonorrhoea (P = 0·001 for both).

Previous experience of urethritis had only limited effect in shortening the interval between infection and attendance. Compared with those with a first episode of urethritis it had no effect on the incubation periods but did shorten the duration of symptoms. "Anxiety" was of greater benefit; possibly because of their increased awareness these patients noticed the symptoms of both conditions earlier, especially those with NSU. Its beneficial effect on the duration of symptoms was confined to those with gonorrhoea, possibly because the latter is a more painful condition than NSU.

The most important factor in determining the interval between infection and attendance, however, was whether or not the patient knew the contact. As might be expected in a specific infection knowledge of the contact did not prolong the incubation period of gonorrhoea for any of the three groups, but it had a profound effect on the duration of symptoms. This was also true of patients with NSU, but in addition the incubation period of those with a first episode of urethritis was significantly affected.

Thus the spectrum of initial infections of NSU may possibly have differed between the two groups, those acquired from strangers being the more virulent and having shorter incubation periods and duration of symptoms than those acquired from friends. We could not confirm this, because in 1978 we did not have a chlamydial culture service (*Chlamydia trachomatis* being the most likely organism to be acquired from casual contacts). Such a service may not have been of help, however, as Oriel *et al*[6] had noted that the mean incubation period of urethritis in patients harbouring chlamydia (16 days) was longer than in those who did not (12 days). Furthermore, most authors[7-9] have reported the mean duration of symptoms of acute NSU to be about 30% longer when chlamydia had been isolated than when it had not. Only one author reported a marginally shorter mean duration of symptoms in the presence of chlamydia.[10]

Willcox[11-13] found an association between the proportion of known contacts with initial episodes of urethritis and the length of the mean incubation

TABLE III *Gonorrhoea and non-specific urethritis in men. Mean (± SEM) incubation periods and durations of symptoms by type of attendance and knowledge of contact (in days)*

		No (%) of patients with:		*Mean (± SEM) incubation periods (days):*		*Mean (± SEM) duration of symptoms (days):*		*Total periods*	
Attendance groups	*Contact*	*Gonorrhoea*	*NSU*	*Gonorrhoea*	*NSU*	*Gonorrhoea*	*NSU*	*Gonorrhoea*	*NSU*
A: initial urethritis	Known	59 (24·4)	59 (22·4)	7·1 ± 4·3	10·1 ± 4·1	5·3 ± 2·2	6·8 ± 4·5	12·4	16·9
A: initial urethritis	Unknown	119 (49·2)	92 (34·8)	6·4 ± 3·8	7·0 ± 2·9	2·4 ± 1·4	2·9 ± 1·6	8·8	9·9
B: repeated urethritis	Known	16 (6·6)	38 (14·4)	6·7 ± 4·1	8·4 ± 3·9	3·6 ± 1·5	5·3 ± 3·7	10·3	13·7
B: repeated urethritis	Unknown	30 (12·4)	58 (22·0)	4·8 ± 2·6	6·7 ± 4·7	1·9 ± 1·0	2·2 ± 1·6	6·7	8·9
C: no previous abnormality	Known	3 (1·2)	9 (3·4)	3·3 ± 0·8	4·9 ± 3·1	3·0 ± 0·0	4·3 ± 3·9	6·3	9·2
C: no previous abnormality	Unknown	15 (6·2)	8 (3·0)	4·6 ± 2·9	5·3 ± 3·4	1·3 ± 0·7	2·1 ± 1·1	5·9	7·4
Totals		242 (100·0)	264 (100·0)	6·2 ± 3·8	7·7 ± 4·1	3·1 ± 2·3	4·0 ± 3·4	9·3	11·7

SEM = standard error of mean

periods, as in this study. The overall longer mean duration of symptoms (3·9 days) could have been associated with the higher proportion of known contacts (49%) compared with the 32% reported here.

In one report on patients with NSU[14] the mean incubation period was longer than 9·5 days and the duration of symptoms more than 9·0 days compared with 7·7 and 4·0 days respectively in this study. A similar proportion (57%) had an initial episode of urethritis, but 72% had known contacts compared with only 40% in this study; this may explain the differences in the incubation periods and durations of symptoms.

While those with initial episodes of urethritis and known contacts have been identified as groups at special risk of having prolonged symptoms, the benefit of having a repeat episode of urethritis was minimal. Anxious patients and those with unknown contacts were rarely at risk, and, when combined, the maximum duration of symptoms for gonorrhoea was three days and for NSU four days. Symptoms were prolonged (eight days or more) in five (2·1%) of those with gonorrhoea and in 21 (8·0%) of those with NSU; all were associated with known contacts. Of those with gonorrhoea, three had repeat episodes of urethritis and two first episodes. Among those with NSU 16 had first episodes of urethritis, four repeat episodes, and one no abnormality detected on previous attendance. Conversely, extremely short durations of symptoms were all associated with unknown contacts. Six (2·5%) men with gonorrhoea, but none with NSU, attended within a day of noticing symptoms, five having no abnormality on previous attendance and one a repeat episode of urethritis.

Patients, therefore, need to be more generally aware that known contacts may be a source of infection, even when the relationship is stable, and that the period of reconciliation after separation is especially dangerous. The public also need to be educated away from the attitude of "it can't happen to me." In the clinics all patients, with or without urethritis, should be educated to attend for routine examination after contact with strangers, or as soon as symptoms develop, but not to indulge in persistent self-examination. Finally, clinicians should always examine carefully even the most persistent of "neurotic" patients, because they may become infected eventually.

References

1. King A, Nicol CS, Rodin P. *Venereal Diseases,* 4th ed. London: Baillière and Tindall, 1980:199.
2. Catterall RD. *A Short Textbook of Venereology.* 2nd ed. London: English Universities Press, 1974:16.
3. Morton RS. *Gonorrhoea.* London: WB Saunders, 1977:70.
4. Robertson DHH, McMillan A, Young H. *Clinical Practice in Sexually Transmitted Diseases.* Tunbridge Wells: Pittman Medical, 1980:167.
5. Schofield CBS. *Sexually Transmitted Diseases.* 3rd ed. Edinburgh: Churchill Livingstone, 1979:135.
6. Oriel JD, Reeve P, Powis P, Miller A, Nicol CS. Isolation of chlamydia from patients with non-specific genital infection. *Br J Vener Dis* 1972;**48**:429-36.
7. Alani MD, Darougar S, MacD Burns DC, Thin RN, Dunn H. Isolation of *Chlamydia trachomatis* from the male urethra. *Br J Vener Dis* 1977;**53**:88-92.
8. Perroad HM, Miedzybrodzka K. Chlamydial infection of the urethra in men. *Br J Vener Dis* 1978;**54**:45-9.
9. Terho P. *Chlamydia trachomatis* in non-specific urethritis. *Br J Vener Dis* 1978;**54**:251-6.
10. Ghadirian FD, Robson HG. *Chlamydia trachomatis* genital infections. *Br J Vener Dis* 1979;**55**:415-8.
11. Willcox RR. Effective treatment of gonorrhoea in London with two oral doses of amoxycillin. *Br J Vener Dis* 1974;**50**:120-4.
12. Willcox RR. Spectinomycin in the treatment of gonorrhoea in males. *Br J Vener Dis* 1974;**50**:294-7.
13. Willcox RR. Single oral dose of 1·5 g talampicillin in the treatment of gonorrhoea. *Br J Vener Dis* 1976;**52**:184-6.
14. Willcox RR, Sparrow RW, Fazluddin CM. Minocycline in the treatment of non-gonococcal urethritis. *Br J Vener Dis* 1975;**51**:206-9.

Br J Vener Dis 1982;**58:**188-95

Ultrahistopathology of balanitis circinata

LASSE KANERVA,* MERJA KOUSA, KIRSTI-MARIA NIEMI, ALLAN LASSUS, TIMO JUVAKOSKI, AND JORMA LAUHARANTA

*From the Departments of *Dermatology, Anatomy, and Electron Microscopy, University of Helsinki, Helsinki, Finland*

SUMMARY Penile lesions from six patients with balanitis circinata were examined with the light and electron microscopes. The epithelium showed slight parakeratosis, acanthosis, and elongation of rete ridges. Neutrophil pustules occupied the upper epidermis. Prickle cells formed a spongiform net around the pustules.

The most prominent features detected by the electron microscope were the small-to-medium-sized pustules in the upper epidermis. The thin flattened keratinocytes formed a sponge-like trabecular network. Neutrophils were not found inside the keratinocytes. Chlamydia were not present in the lesions, which indicates that balanitis circinata is due to some reactive mechanism. The fine structure of balanitis circinata resembles that of pustular psoriasis.

Introduction

Balanitis circinata is almost diagnostic of Reiter's disease and occurs in up to 69% of male patients with this disease.[1] Similar lesions (vulvitis circinata) have also been found in the female vulva.[2]

The spongiform pustules of the skin are the most distinctive histopathological finding of Reiter's disease detected by a light microscope. These pustules may be seen in cutaneous, oral, and penile circinate lesions.[3-5] Hyperkeratosis, parakeratosis, and acanthosis may also be present in cutaneous lesions, which resemble those of psoriasis.

Descriptions of the fine structure of balanitis circinata have not been reported. For this reason we decided to undertake the present study.[6]

Materials and methods

Six patients with balanitis circinata were included voluntarily in the study. Their clinical and laboratory findings are given in the table. Gonorrhoea was excluded by smear examination and negative culture results for *Neisseria gonorrhoeae* in all patients. Cultures for *Chlamydia trachomatis* were performed on urethral specimens in all cases; in two patients the results were positive, in two who had had a previous episode of chlamydial urethritis they were now negative, and in two who had not had chlamydial urethritis previously they were negative.

TABLE *Details of six patients with balanitis circinata*

Patient No	*Age (years)*	*Clinical symptoms*	*HLA-B27*	*Chlamydial isolation*
1	24	Urethritis, arthritis	+	–
2	22	Urethritis, arthritis	+	+
3	25	Urethritis, arthritis, nail changes	ND	+*
4	52	Arthritis	+	–
5	23	Urethritis, arthritis	+	+*
6	40	Urethritis	ND	+

+ = Positive; – = negative; ND = not done
*Isolation-positive at the time of examination

Samples for conventional light microscopy (one patient) and for light and electron microscopy (all patients) were taken from the circinate lesion of the glans penis with a knife after local anaesthesia with 1% lidocain-adrenaline. For electron microscopy, blocks (1 mm³ or smaller) were fixed for two hours or longer in cacodylate or phosphate-buffered (0·1 mmol/l; pH 7·3) 2·5% glutaraldehyde at 4°C, postfixed with 1% osmium tetroxide, dehydrated in graded series of alcohol, and embedded in Epon 812. Ultrathin sections were cut with a diamond knife on a LKB ultramicrotome, stained with lead citrate, and examined with a Jeol 100S or a Jeol 100CX electron microscope operated at 80 kV.

Results

CLINICAL

The penile lesions in all patients were typical of balanitis circinata. They showed small erythematous

Address for reprints: Dr L Kanerva, Department of Dermatology and Venereology, University Central Hospital, Snellmaninkatu 14, 00170 Helsinki 17, Finland

Accepted for publication 21 December 1981

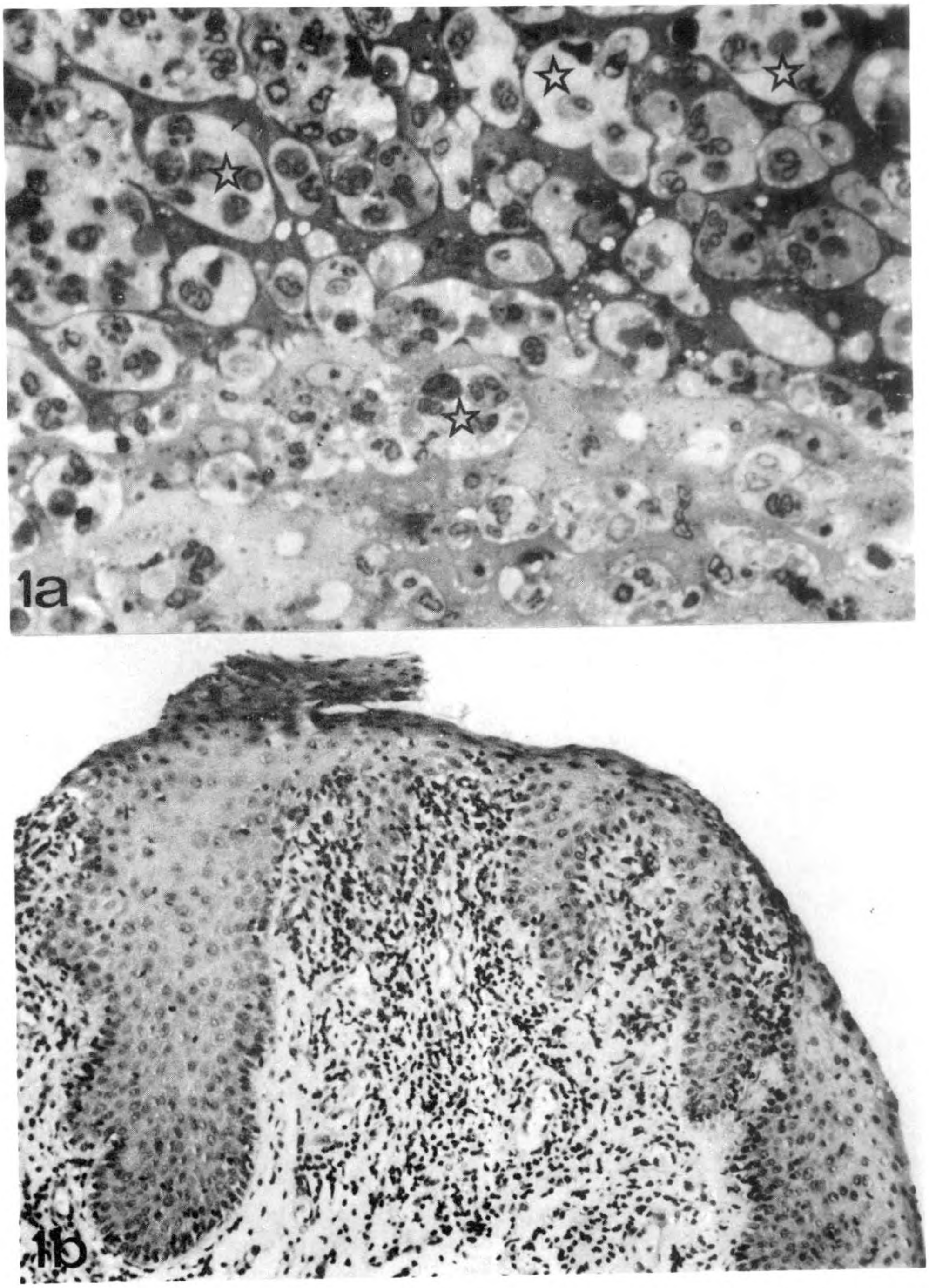


FIG 1 *Histopathology of balanitis circinata by light microscopy. (a) Neutrophil pustules (star) occupy the upper epidermis. (Epon embedment,* × *940). (b) Parakeratosis, acanthosis, and elongation of rete ridges and only a few neutrophils are seen, but an extensive mononuclear infiltrate is present. (Paraffin embedment, patient 6,* × *210).*

areas, which were demarcated from the surrounding normal mucosa by shallow, circinate or serpiginous, slightly raised, greyish white borders.

LIGHT MICROSCOPY

The epithelium showed slight parakeratosis, acanthosis, and elongation of rete ridges. Neutrophil pustules occupied the upper epidermis (fig 1*a*). Prickle cells formed a spongiform net around the pustules. The basal epidermis was invaded by a moderate number of mononuclear cells. The keratinocytes were moderately oedematous and the intercellular space enlarged.

In the papillary dermis the capillaries were enlarged and increased in number. In one patient, a moderate-to-extensive pericapillary mononuclear infiltrate was present (fig 1*b*). Some erythrocyte extravasation was present.

ELECTRON MICROSCOPY

In the upper dermis fenestrated (fig 2) or unfenestrated capillaries were widened and surrounded with neutrophils and mononuclear cells of the lymphocytic and macrophagic cell lines. Melanophages were also present. The dermoepidermal junction had a normal appearance with well preserved hemidesmosomes (fig 2), although basal lamina gaps were sometimes found. Leucocytes invaded the epidermis through these gaps.

The keratinocytes showed increased cellular activity with numerous ribosomes, mitochondria, Golgi areas, and endoplasmic reticulum and also

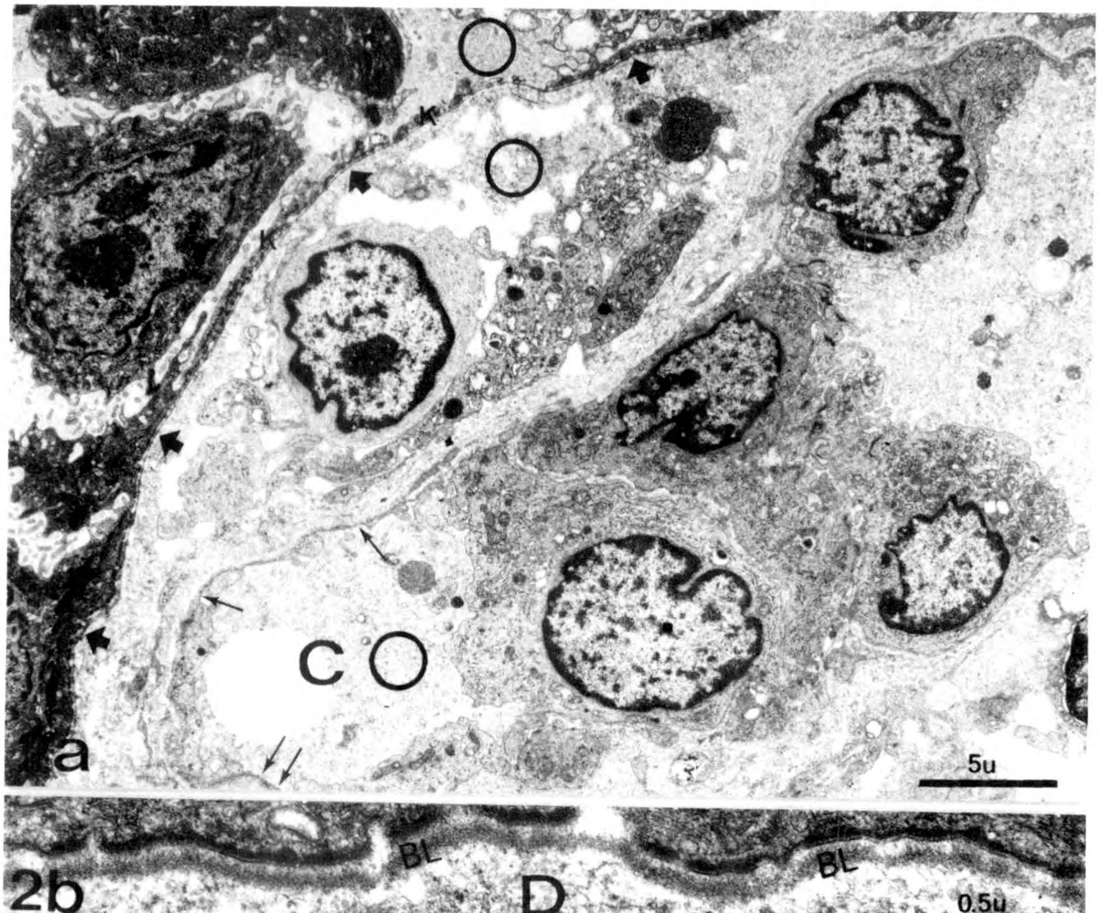


FIG 2 *Electron micrograph of the dermoepidermal junction. (a) Fenestrated (thin arrow) capillary (C) lies close to the basal lamina (thick arrow). Note the flattened basal keratinocytes (K). A proteinous, fine, granular substance is present in the capillary, in the papillary dermis, and in the epidermis (circle) (× 3600). (b) Prominent long band of hemidesmosomes is shown. (BL = basal lamina; D = dermis, × 36 000).*

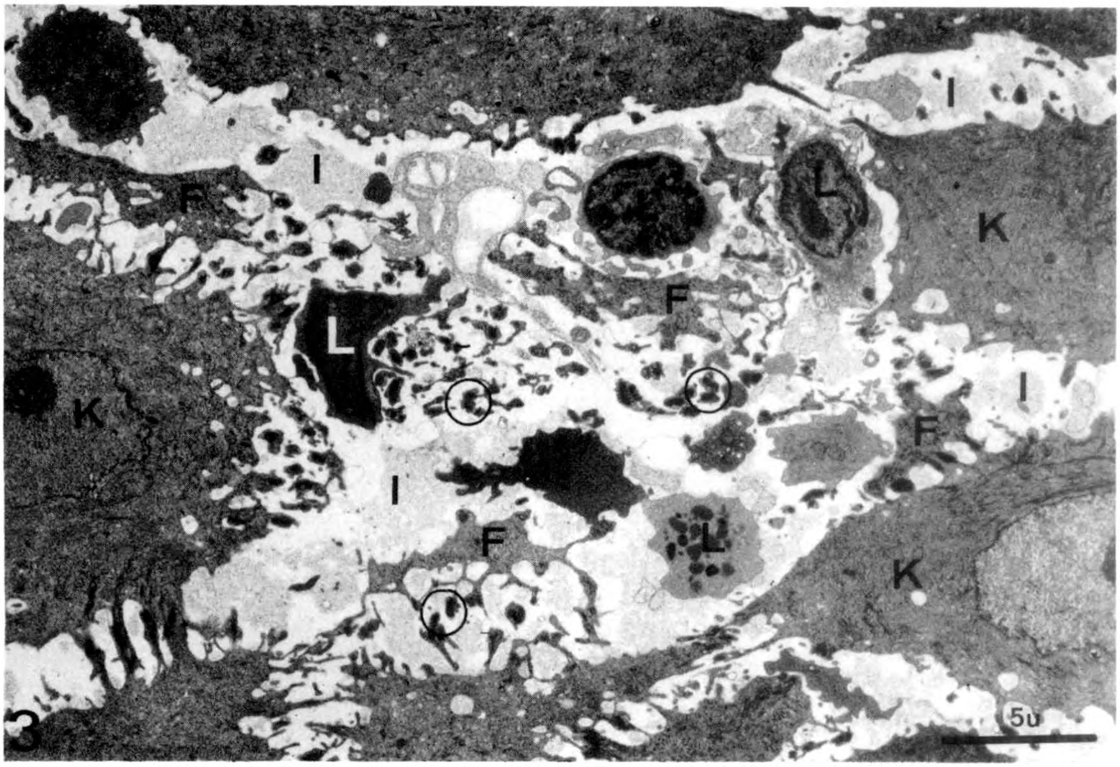


FIG 3 *Electron micrograph showing prickle cell layer with widened intercellular space (I) invaded by leucocytes (L). Fewer tonofilaments in the keratinocytes (K), some of which are flattened (F), and distended desmosomes (circle) are present. (× 4000).*

increased numbers of lipid droplets. Giant mitochondria were found frequently in the superficial keratinocytes, some of which were oedematous. There were few tonofilaments. The intercellular space was enlarged and the number of desmosomes decreased (fig 3). The substance in the intercellular space was finely granular as was that inside and around the capillaries, indicating leakage of plasma through the capillaries (fig 4). A coarse granular substance, probably derived from keratinocyte organelles, was also found. Large dark bodies (fig 4) without a visible membrane and with a heterogeneous inner structure were often present in the middle and upper epidermis; these may have been lipid bodies.[7]

The most prominent feature was the neutrophils, which formed small-to-medium-sized pustules in the interstices (figs 3 and 4); this caused flattening of the keratinocytes. The thin flattened keratinocytes formed a sponge-like trabecular network connected by a few tiny desmosomes. In addition to neutrophils, free lysosomes and other organelles from disrupted neutrophils and possibly keratinocytes were present in the intercellular space in these pustules. Often the surface membrane of contacting keratinocytes and neutrophils was partly absent (fig 5), allowing the passage of lysosomes from the neutrophils into the adjacent keratinocyte. These lysosome-containing keratinocytes were otherwise normal in appearance and did not show prominent features of cytolysis. Neutrophils were not found inside the keratinocytes. Neutrophil pustules were usually present in the superficial layers, corresponding to a "subcorneal" localisation. Lymphocytes occurred rarely in the superficial pustules whereas large numbers of lymphocytes and macrophages were present in the basal layers. Chlamydia were not found in the lesions.

These findings were almost identical in five of the patients. In the sixth patient (patient 6), the invading leucocytes were mostly of the lymphocyte-macrophage series, with only a few neutrophils (fig 1*b*), and the pericapillary infiltrate of the dermis was dense. The mononuclear cells were in very close contact with the basal lamina and frequently invaded the epidermis through gaps in it (fig 6).

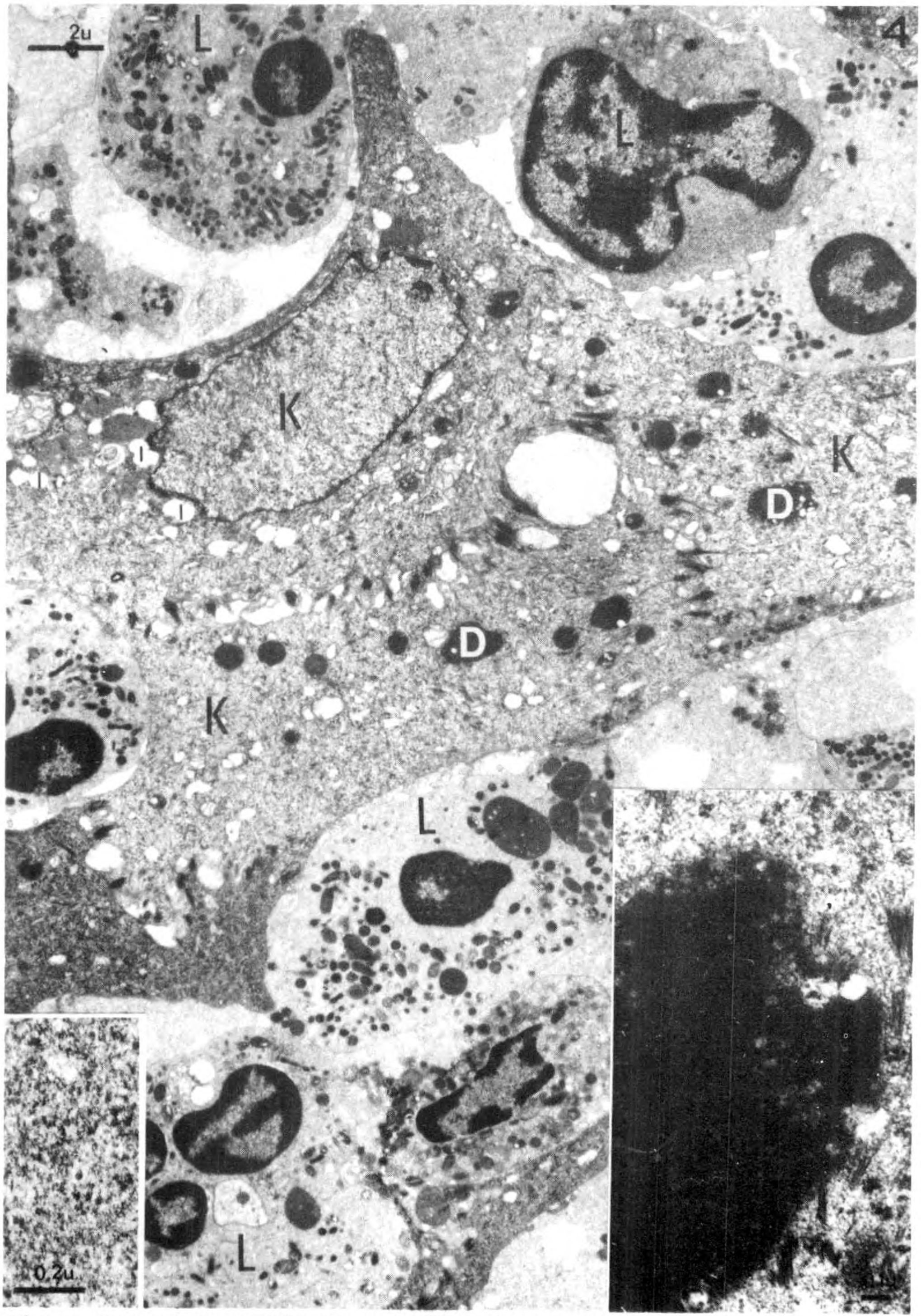


FIG 4 *Electron micrograph showing infiltrating neutrophils (L) between keratinocytes (K) containing abundant lipid droplets (l) and dark bodies (D) (× 6000). Left inset: Fine granular intercellular substance at larger magnification (× 60 000). Right inset: Dark body at larger magnification (× 45 000).*

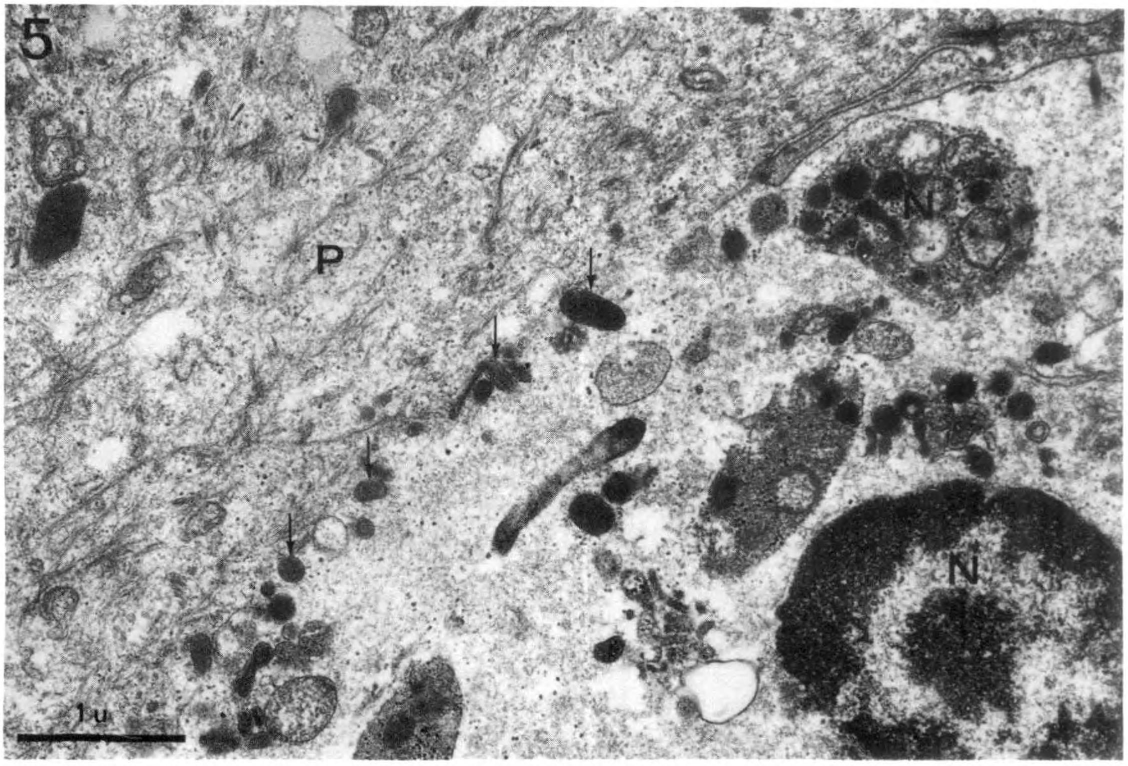


FIG 5 *Electron micrograph showing that surface membranes of contacting neutrophils (N) and prickle cell (P) have partly disappeared allowing passage of leucocyte granules (arrow) into prickle cell (× 22 000).*

Discussion

Reiter's disease has been defined as a disorder of unknown cause consisting of urethritis, arthritis, and conjunctivitis, accompanied in some cases by uveitis, circinate balanitis, and oral lesions, and in rare cases by an eruption simulating rupioid psoriasis (keratodermia blenorrhagica).[8]

Two distinct infections appear to be the triggering factor in Reiter's disease, namely enteritis and nongonococcal urethritis (NGU), which is now the most frequent factor in cases in developed countries.[9] *C trachomatis* can be isolated from the urethral tract of about 70% of men with Reiter's disease who show signs of urogenital infection at the time of examination.[10] In Finland, approximately 2% of men with NGU develop signs of polysymptomatic Reiter's disease.[10] The histocompatability antigen HLA-B27 is strongly associated both with Reiter's disease[11] and with balanitis circinata.[12] Thus, balanitis circinata may be an early sign of Reiter's disease, even when it occurs alone.[9]

Keratodermia blenorrhagica of the palms or soles or both has been reported in 1-18%[1] of patients with Reiter's disease and in about one-fifth of patients with other psoriasis-like dermatoses.[1] Our finding of spongiform pustules in the circinate lesions further confirmed the strong relationship between the skin lesions of Reiter's disease and psoriasis. At the fine structural level the spongiform pustules closely resembled those reported in pustular psoriasis[7] and impetigo herpetiformis.[13] Our finding of close interactions between infiltrating neutrophilic leucocytes and keratinocytes has also been reported by Komura *et al.*[13] The cell membranes of contacting leucocytes and keratinocytes were occasionally partly absent, and the leucocyte granules appeared to move into the adjacent keratinocyte.[13] The cytolytic enzymes of the neutrophil granules[14] probably accounted for the generation of the sponge-like network of keratinocytes. Our study further confirmed that the neutrophils were localised between[7 13] and not within the keratinocytes, as was believed during the light microscopy era.

Little is known about the pathogenesis of the skin lesions in Reiter's disease. The basic mechanisms may be closely related in several diseases which are characterised by epidermal non-bacterial pustules, such as pustular psoriasis, impetigo herpetiformis, acrodermatitis continua Hallopeau, pustulosis

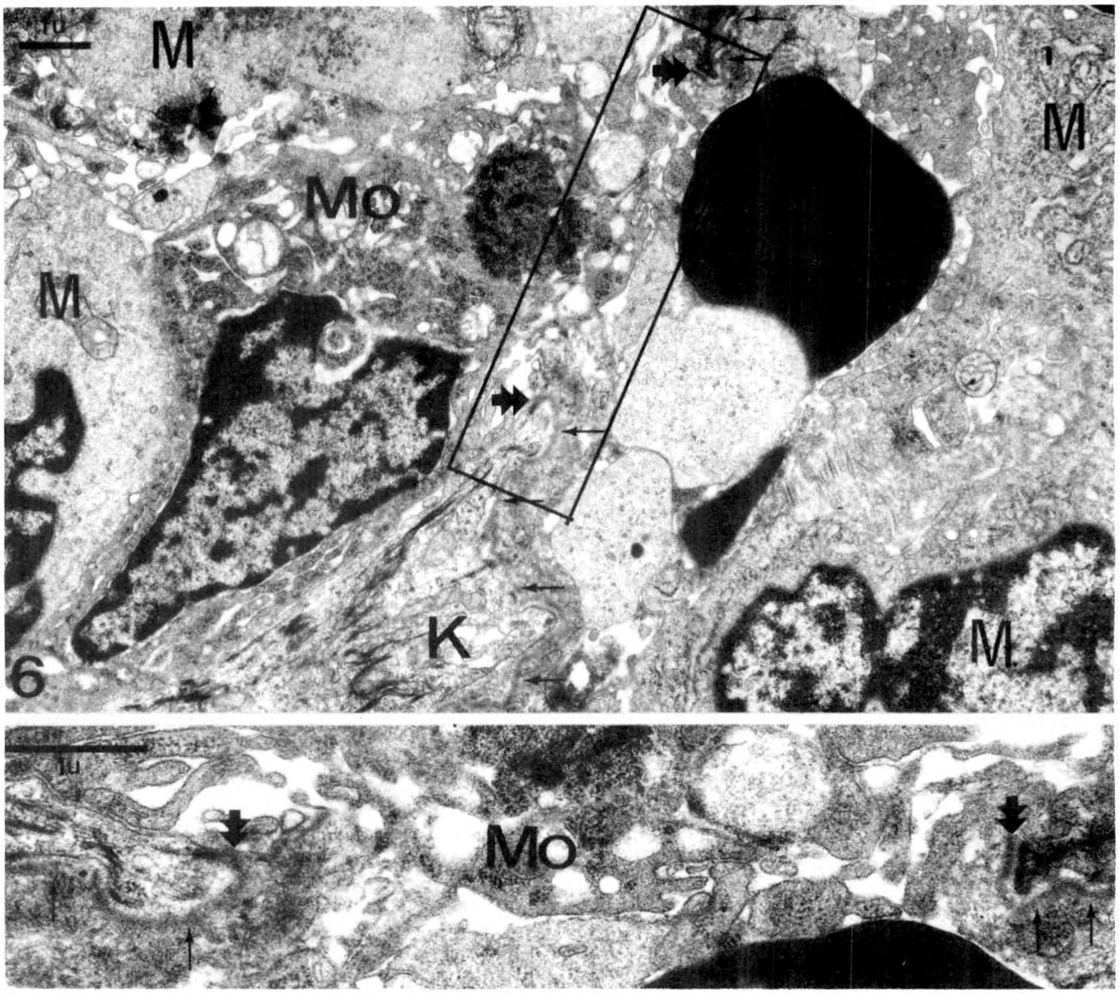


FIG 6 *Electron micrograph showing dermoepidermal junction with very large number of mononuclear cells (M) in the upper dermis (patient 6). One mononuclear cell (Mo) is penetrating the dermoepidermal junction through a gap in the basal lamina (between double-headed arrows). (Arrow indicates basal lamina; K = basal keratinocyte, × 9000). Lower section shows framed area at larger magnification (× 19 000).*

palmoplantaris, geographic tongue,[15] and Reiter's disease. The clinical course and morphological appearance of the circinate lesions which are present in Reiter's disease and psoriasis as well as in leprosy, dermatophytosis, certain varieties of dermatitis, and cutaneous lymphoma indicate an immunological pathogenesis.[16] Our morphological findings indicated that immunological factors played a part in pustule formation, as previously suggested in pustulosis palmoplantaris[17] and psoriasis.[16] We found dilated capillaries with polymorphonuclear leucocytes, basal lamina gaps, basal spongiosis, and pustules of polymorphonuclear leucocytes. Complement-dependent chemotactic factors[18 19] have been identified in psoriatic lesions and may be present in the skin and mucous lesions of Reiter's disease. These could cause an accumulation of polymorphonuclear leucocytes which release various cytotoxic enzymes[14] and thus account for the keratinocyte damage. The dilated fenestrated papillary capillaries would discharge the polymorphonuclear leucocytes, which pass through the basal lamina gaps, forming the proximal Kogoj pustules and the more distal Munro microabscesses. Why the penile mucosa is such a characteristic target organ in Reiter's disease is not known, but balanitis circinata seems to occur almost solely in patients with Reiter's disease triggered by a urogenital infection. The absence of chlamydia in the lesions suggests that balanitis circinata is due to some reactive mechanism.

References

1. Kousa M. Reiter's disease. A clinical, microbiological and HLA study. Doctoral thesis. Helsinki, Finland, 1978.
2. Thambar IV, Dunlop, R, Thin RN, Huskisson EC. Circinata vulvitis in Reiter's syndrome. *Br J Vener Dis* 1977;**53**:260-2.
3. Kulka P. The lesions of Reiter's syndrome. *Arthritis Rheum* 1962;**2**:195-201.
4. Weinberger HW, Ropes MW, Kulka JP, Bauer W. Reiter's syndrome, clinical and pathological observations. A long-term study of 16 cases. *Medicine (Baltimore)* 1962;**41**:35-91.
5. Pindborg JJ, Gorlin RJ, Asboe-Hansen G. Reiter's syndrome. Review of the literature and report of a case. *Oral Surg* 1963; **16**:551-60.
6. Kanerva L, Kousa M, Niemi K-M, Juvakoski T, Lassus A. Ultrastructure of balanitis circinata. In: International Society for STD Research, Fourth International Meeting on Sexually Transmitted Diseases, Heidelberg, October 18-20 1981. *Abstracts* 1982:72.
7. Rupec M. Zur Ultrastruktur der spongiformen Pustel. *Arch Klin Exp Dermatol* 1970;**239**:30-49.
8. Lockie GN, Hunder GG. Reiter's syndrome in children. A case report and review. *Arthritis Rheum* 1971;**14**:767-72.
9. Lassus A, Kousa M, Juvakoski T, Kanerva L, Lauharanta J. Balanitis circinata and *Chlamydia trachomatis*. In: Abbott Laboratories, ed. International Symposium on Chlamydia, Belgium, Antwerp, October 2-3, 1980. *Abstracts* 1981:31-40.
10. Kousa M, Saikku P, Richmond S, Lassus A. Frequent association of chlamydial infection with Reiter's syndrome. *Sex Transm Dis* 1978;**5**:57-61.
11. Aho K, Ahvonen P, Lassus A, Sievers K, Tiilikainen A. HLA-A 27 in reactive arthritis. A study of yersinia arthritis and Reiter's disease. *Arthritis Rheum* 1974;**17**:521-5.
12. Lassus A, Tiilikainen A, Stubb S, Reunala T, Johansson E. Circinate erosive balanitis and HLA-A 27. *Acta Derm Venereol (Stockholm)* 1975;**55**:199-201.
13. Komura J, Masahiro T, Shigeo O. Epidermal cell-leucocyte interactions in spongiform pustules of Kogoj. An ultrastructural study. *Arch Dermatol Res* 1974;**249**:321-8.
14. Olsson I, Venge P. The role of the human neutrophil in the inflammatory reaction. *Allergy* 1980;**35**:1-13.
15. Dawson TAJ. Tongue lesions in generalized pustular psoriasis. *Br J Dermatol* 1974;**91**:419-24.
16. Cormane RH. Views and concepts. Immunopathology of psoriasis. *Arch Dermatol Res* 1981;**270**:201-15.
17. Rajka G. Immunology of pustulosis palmoplantaris. *Acta Derm Venereol (Stockholm)* 1979;**87**:31-2.
18. Tagami H, Ofuji S. Leukotactic properties of soluble substances in psoriasis scale. *Br J Dermatol* 1976;**95**:1-8.
19. Lazarus G, Yost FJ, Thomas ChA. Polymorphonuclear leucocytes: possible mechanism of accumulation in psoriasis. *Science* 1977;**198**:1162-3.

Br J Vener Dis 1982;**58:** 196-9

Rheumatoid arthritis, ankylosing spondylitis, and Reiter's syndrome occurring simultaneously

Case report

H A LAVERY,* T HORNER,* AND S D ROBERTS†

*From the Departments of *Genitourinary Medicine and †Rheumatology, Royal Victoria Hospital, Belfast, Northern Ireland*

SUMMARY Features of rheumatoid arthritis, ankylosing spondylitis and Reiter's disease occurred simultaneously in the same patient. This is a rare finding and appears to be the first published report of such a case.

Case report

A 60-year-old man presented at the genitourinary medicine clinic in December 1980 complaining of a clear urethral discharge and dysuria. His past medical history included a back injury in 1940, urethritis diagnosed and treated in the Royal Navy in 1943, lobectomy for tuberculosis in 1949, non-specific urethritis diagnosed and treated in the Royal Victoria Hospital in 1958, ankylosing spondylitis diagnosed in the same hospital in 1967, iritis in 1968, and painful swelling and stiffness of the joints of both hands and feet in 1976. There was no family history of arthropathy.

On examination he was alert and cheerful. There was a right thoracotomy scar. A clear mucoid urethral discharge was present. Using the criteria that if pus cells fill one quadrant of the microscopic field the result is pus cells + and if four quadrants are filled the result is pus cells + + + +, this patient had pus cells + + +. No *Neisseria gonorrhoeae* were observed or cultured. There were rheumatoid deformities of the hands, ulnar deviation of the fingers, and subcutaneous nodules at each elbow. Spinal flexion was limited, the fingertip-to-ground distance was seven inches, rotational movements were very restricted, and chest expansion was one inch. No iritis, conjunctivitis, or rash was present. He was treated with oxytetracycline 250 mg, one tablet four times daily.

One week later he complained of sudden painful swelling of both feet and a tender right testis. On examination he had circinate balanitis (fig 1) and right epididymo-orchitis. The joints of both feet and hands were acutely swollen and painful, allowing only a little passive movement.

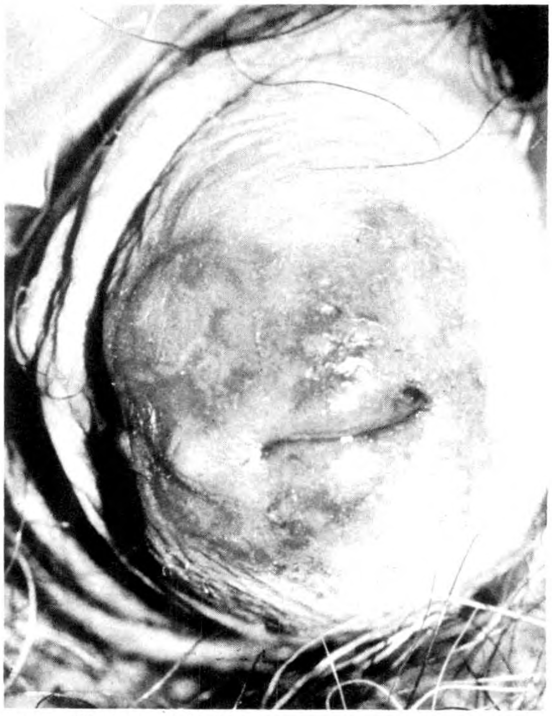


FIG 1 *Circinate balanitis.*

On admission to hospital initial investigations showed an erythrocyte sedimentation rate of 115 mm /first hour, which one week later had risen to 130 mm/first hour; Hb was 11·2 g/dl (normal 12-18 g/dl), mean corpuscular haemoglobin 25·1 pg (normal 27-32 pg), and a white cell count 12·7 ×

Address for reprints: Dr H A Lavery, Department of Genitourinary Medicine, Royal Victoria Hospital, Belfast BT12 6BA, Northern Ireland

Accepted for publication 12 December 1981

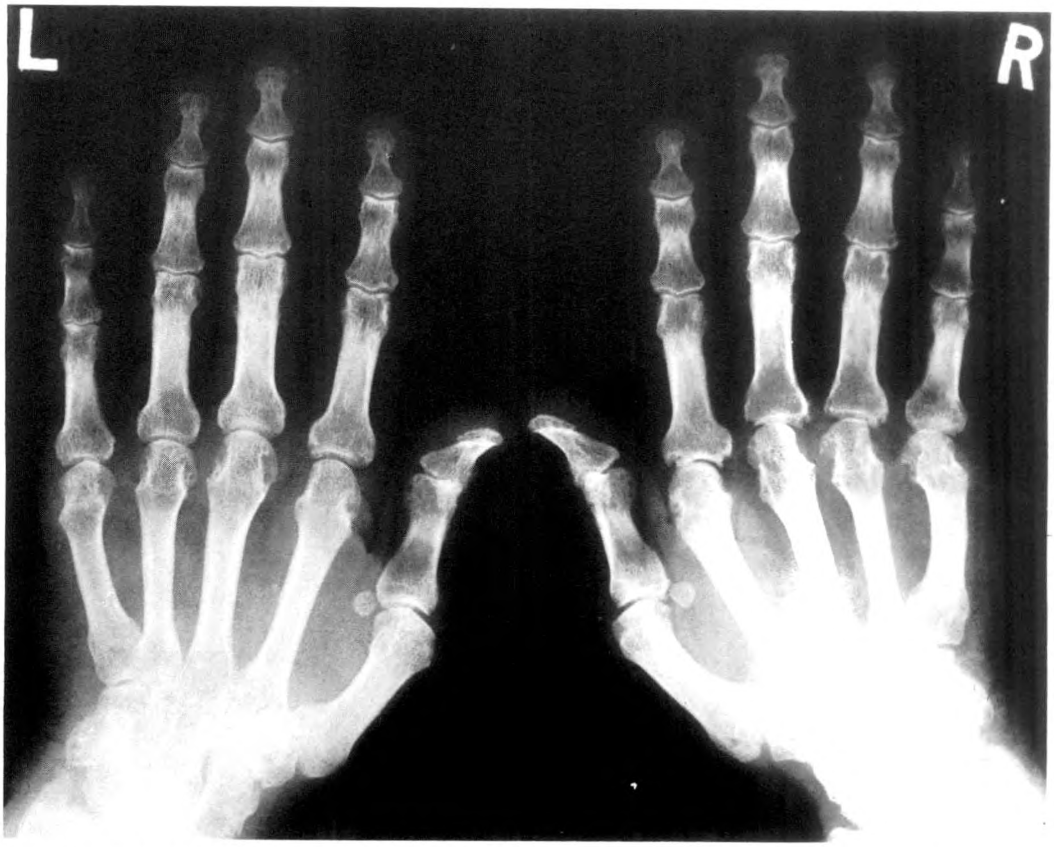


FIG 2 *Radiograph of right and left hand showing multiple erosions particularly in the metacarpophalangeal joints.*

10^9/l (normal 4-11 × 10^9/l). The urinary excretion of hydroxyproline was 0·9 mmol/24 h (117·9 mg/24h) (normal range 0·11-0·35 mmol/24 h; 14·4-45·8 mg/24h). Chlamydial antibodies were not present in the serum by the lymphogranuloma venereum complement-fixation test. No culture for *Chlamydia trachomatis* was undertaken but cultures for urinary pathogens gave negative results. IgM rheumatoid factors were detected by the rheumatoid arthritis latex test using pooled human gammaglobulin-coated latex particles (Hyland), and the Rose-Waaler differential agglutination titre was 8 (normal ⩽16); HLA-B27 was present. Radiographs showed multiple articular erosions in both hands (fig 2) and in the right medial malleolus. The lumbar spine and sacroiliac joints showed typical advanced ankylosing spondylitis (fig 3). A biopsy (fig 4) of the nodule from the right elbow showed foci of fibrinoid necrosis with some palisading of histiocytes characteristically seen in rheumatoid disease.

He was treated with rest in bed, oral iron, azapropazone, and naproxen. The haematological indices returned to normal, the ESR fell to 60 mm/first hour, and he was symptomatically improved on discharge one month later.

Discussion

Rheumatoid arthritis, with chronic deforming symmetrical arthritis, radiological erosions, and histologically confirmed nodules, was present in this patient. The association of ankylosing spondylitis and rheumatoid arthritis is rare[1]; in the Mayo Clinic series where 7135 patients with rheumatoid arthritis and 1162 patients with ankylosing spondylitis attended from 1970-74, the diseases occurred simultaneously in only two cases. Statistically, they would occur together by chance in 1 in 50 000 to 1 in 200 000 cases.[2] The diagnosis of ankylosing spondylitis in this man was based on the findings of limitation of spinal movements in two planes, bilateral sacroiliitis, and typical vertebral ankylosis. Prostatitis may be present in 80% of patients with ankylosing spondylitis.[3] In our patient pus cells + + were observed after

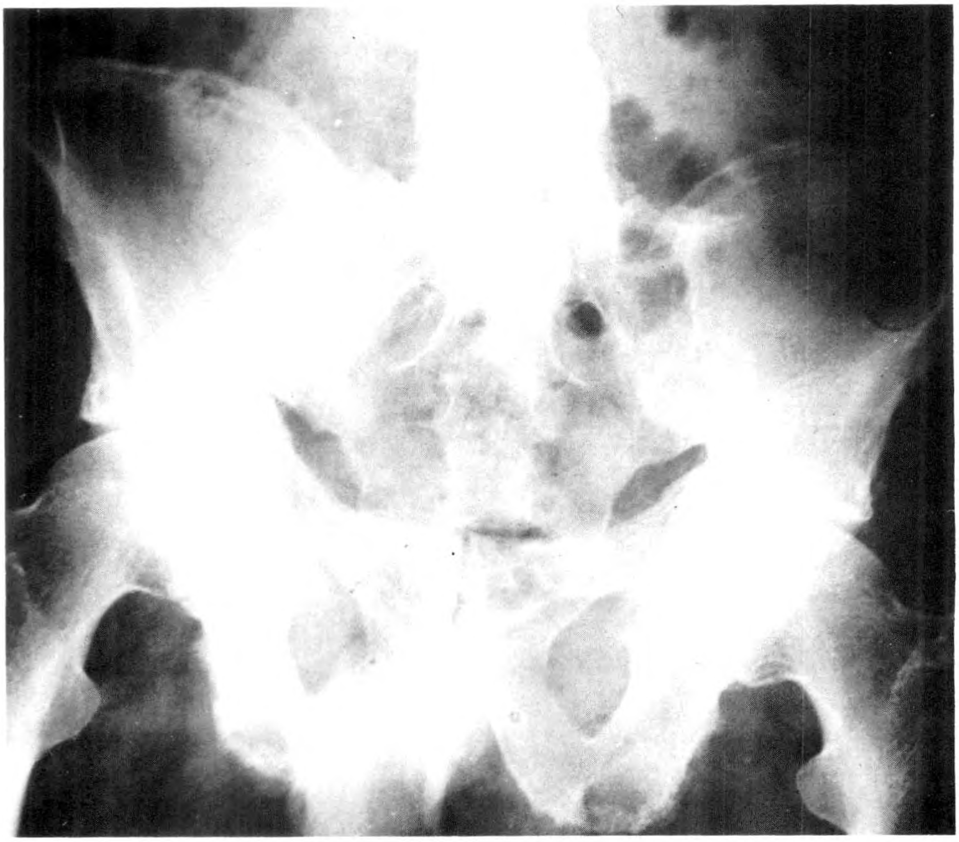


FIG 3 *Radiograph of sacroiliac joints and lumbar spine showing bilaterial sacroiliitis and vertebral ankylosis.*

prostatic massage. A diagnosis of Reiter's syndrome was made in view of the circinate balanitis, epididymo-orchitis, and acute arthropathy superimposed on the previous arthritis. Rheumatoid arthritis may be associated with Reiter's disease and a family incidence has been reported. Good[4] described a family of three brothers, one of whom had Reiter's disease followed by rheumatoid arthritis, one had Reiter's disease followed by ankylosing spondylitis, and one Reiter's disease; their father had rheumatoid disease. There was no family history in this case, which appears to be the first report of ankylosing spondylitis, rheumatoid arthritis, and features of Reiter's syndrome occurring in the same patient.

References

1. Lathra HS, Ferguson RH, Conn DL. Co-existence of ankylosing spondylitis and rheumatoid arthritis. *Arthritis Rheum* 1976; **19:** 111-4.
2. Robertson DHH, McMillan A, Young H. *Clinical Practice in Sexually Transmitted Diseases.* 1st ed. Kent: Pitman Medical, 1980: 238-63.
3. Fallet GH, Mason M, Berry H, Mowat A, Boussina I, Gerster J-C. Rheumatoid arthritis and ankylosing spondylitis occurring together. *Br Med J* 1976; i: 804-7.
4. Good AE. Reiter's disease, ankylosing spondylitis and rheumatoid arthritis occurring within a single family. *Arthritis Rheum* 1971; **14:** 753-63.

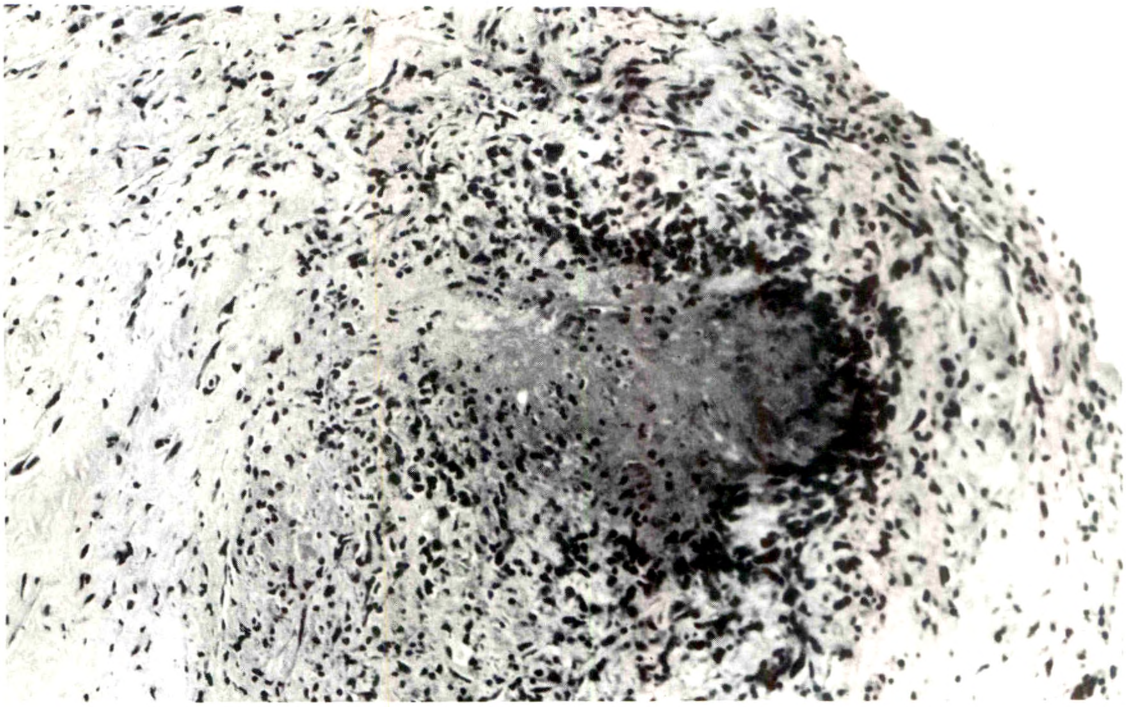


FIG 4 *Section of subcutaneous nodule showing foci of fibrinoid necrosis with some palisading of histiocytes (× 100).*

Br J Vener Dis 1982; **58**: 200-1

Vaginal bleeding in granuloma inguinale

Case reports

S MURUGAN, KAMALAKSHI VENKATRAM, AND P S RENGANATHAN*
From the Department of Venereology, Government Rajaji Hospital, Madurai, Southern India

SUMMARY Since bleeding from the female genitourinary tract is not a common symptom in granuloma inguinale two patients presenting with profuse and intermittent vaginal bleeding were initially admitted as gynaecological or obstetric emergencies. In both cases, the cause was ultimately found to be granuloma inguinale.

Introduction

Granuloma inguinale is a disease known for its extreme chronicity; it does not heal spontaneously and is caused by a fastidious organism *Calymmatobacterium granulomatis*. It is particularly prevalent along the Coramandal coast of the Indian peninsula and occurs almost equally frequently in men and women.

The condition usually presents either with an ulcer or exuberant granulomatous masses or with destruction of the external genitalia due to scarring, commonly in the genitoinguinal, perineal, and occasionally perianal regions. These lesions may be associated with pseudoelephantoid changes of the genitals, expecially in women. As far as we know, bleeding from the female genitourinary tract has not been recorded previously as an important symptom of granuloma inguinale.[1-4]

We describe two patients who were referred to the department of sexually transmitted diseases, Government Rajaji Hospital, Madurai, from the department of obstetrics and gynaecology because of profuse vaginal bleeding and a friable "growth" in the vagina.

Case report (1)

A 33-year-old woman had been married for 19 years and had had five full-term normal pregnancies; all the children were alive and healthy. She complained of a white vaginal discharge, profuse and prolonged menstrual periods for two months, and intermenstrual bleeding. She denied extramarital sexual contact.

CLINICAL FEATURES

The external genitalia and the urethral meatus were normal. The inguinal lymph nodes were just palpable. Speculum examination showed an ulcerative growth affecting the cervix and the anterior, posterior, and lateral walls of the vagina. The ulcer was covered with yellowish-white necrotic material. Moderate bleeding from the ulcers was present. Rectal examination showed a prolapsed pile mass and external piles. There was induration of the posterior vaginal wall and the parametrium was free; the size of the uterus could not be determined.

LABORATORY FINDINGS

Serological tests for syphilis on sera from the patient and her husband gave negative results. Other routine investigations showed no abnormalities. Donovan bodies were found in mononuclear cells in tissue smears taken from the margin of the ulcer.

TREATMENT AND FOLLOW UP

Treatment was started with streptomycin 1 g intramuscularly twice daily. The patient developed giddiness, tingling sensations, and numbness all over the body after the first injection; she refused to have any further injections. Tetracycline 2 g daily in divided doses was given for 20 days (up to a total of 40 g). Her bleeding stopped almost completely.

Speculum examination at the time of discharge showed regression of the ulcer with slight oozing of blood. One month later the ulcers were almost healed and there was no vaginal bleeding. Two months later the patient wrote to us to say that she was well and that her menstrual periods were regular. One year after treatment she was known to be well.

Case report (2)

A 19-year-old woman had been married for 15 months and had had amenorrhoea for seven months;

---

*Deceased

Address for reprints: Dr S Murugan, Department of Venereology, Government Rajaji Hospital, Madurai 625020, Southern India

Accepted for publication 11 December 1981

this was her first pregnancy. After an episode of sudden vaginal bleeding, which was diagnosed as an antepartum haemorrhage by a general practitioner, she was referred to the department of obstetrics and then to this department as she had an intravaginal granulomatous ulcer.

She denied any extramarital contact and had no pain or bleeding during coitus.

CLINICAL FEATURES

On examination the uterus was 24 weeks in size, the inguinal lymph nodes were just palpable, and the external genitalia and the urethral orifice were normal. Speculum examination showed a pink granulomatous cauliflower-like growth on the anterior vaginal wall and close to the anterior lip of the cervix; the posterior lip was healthy. The growth bled profusely when touched.

LABORATORY FINDINGS

Serological tests for syphilis gave negative results on sera from the patient and her husband. Mature capsulated forms of Donovan bodies were found in tissue smears taken from the margin of the ulcer. Histopathological examination showed a granulomatous lesion with no evidence of malignancy.

TREATMENT AND FOLLOW UP

Streptomycin 1 g was given twice daily for 10 days (up to a total of 20 g). No Donovan bodies were detected on smear examination at the end of the 10-day course. The patient was discharged and reviewed after 15 days, when the bleeding had stopped completely, the growth had regressed considerably, and the ulcers were healing well. A month later the ulcers had almost completely healed and there was no bleeding.

Discussion

In both cases of Donovanosis the striking feature of vaginal bleeding was unusual. It may be mistaken for dysfunctional uterine bleeding because of the age of the patient and type of bleeding. Cervical cancer may present as postcoital and postmenopausal bleeding in older women or as antepartum haemorrhage in pregnant women. A bleeding diathesis can also become manifest as vaginal bleeding. In our patients there was no clinical or laboratory evidence for these conditions. The diagnosis of granuloma inguinale was established beyond doubt by the detection of the causative organism in tissue smears and, in the second patient, was confirmed by biopsy. The response to treatment in both patients was additional proof of the aetiology.

References

1. Rajam RV, Rangiah PN. Donovanosis. *WHO Monograph Series No 24*. Geneva: World Health Organisation, 1954.
2. King AJ, Nicol C, Rodin P. *Venereal Diseases*, 4th ed. London: Baillière Tindall, 1980;268.
3. Morton RS, Harris JRW, eds. *Recent Advances in Sexually Transmitted Diseases*, vol 1. Edinburgh: Churchill Livingstone. 1975;194.
4. Kuberski T. Granuloma inguinale (Donovanosis). *Sex Transm Dis* 1980:7:29-36.

Br J Vener Dis 1982; **58:** 202-3

Traumatic ulceration of the cervix

Case report

NICHOLAS J FIUMARA AND THOMAS CAHN

From the Department of Dermatology and Syphilology, Boston Dispensary, Boston, Massachusetts, USA

SUMMARY A 19-year-old woman with persistent biological false-positive reactions to serological tests for syphilis developed multiple elliptical ulcers of the cervix from the use of a vaginal vibrator. This adds one more aetiological agent to the differential diagnosis of cervical ulceration.

Introduction

A number of adornments, prostheses, and other devices used by male homosexuals can cause varying types of trauma to the genitals.[1] We report here a case of traumatic cervicitis, which superficially resembled multiple chancres but gave persistent biological false-positive reactions to serological tests for syphilis.

Case report

A 19-year-old woman attended the Boston Dispensary skin clinic in February 1981 for routine examination for sexually transmitted diseases (STD). She was intending to live with a new boyfriend and wanted to be sure she had no STD. Clinical examination showed no evidence of early symptomatic syphilis or congenital syphilis. The results of a cervical smear and culture for *Neisseria gonorrhoeae* were negative. A wet preparation also gave negative results for *Trichomonas vaginalis* and *Gardnerella vaginalis.* The rapid plasma reagin (RPR) test for syphilis was reactive at a dilution of 1/2, but the fluorescent treponemal antibody-absorption (FTA-ABS) test was non-reactive. A repeat test gave the same results, which were then regarded as biological false-positive reactions. Surface antigen and antibody tests for hepatitis B gave negative results, as did those for autoimmune disease. She admitted to having injected herself with heroin intravenously at intermittent intervals over the previous two years but was not addicted to the drug.

She next attended six months later because her boyfriend had a genital sore, which was found to be traumatic ulceration from fellatio; his RPR and FTA-ABS test results were non-reactive. Nevertheless, the patient had multiple ulcers on her cervix, which were elliptical rather than "punched out"; the fornices were also red and eroded (fig 1). No adenopathy, labial lesions, or body rash were present. She denied that she or her boyfriend used any vaginal device. Darkfield examination gave negative results, the RPR test was reactive at 1/2, and the FTA-ABS test was non-reactive.

She was treated prophylactically with benzathine penicillin G 2·4 million units and asked to return in a week. At this visit she confessed to using a vaginal vibrator, which moved in a circular motion; this explained the cervical ulcerations. A month later the lesions had healed.

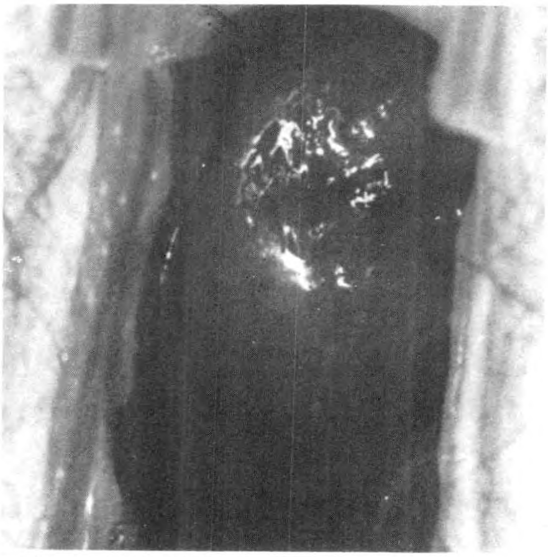


FIG 1 *Ulceration of the cervix*

Address for reprints: Dr N J Fiumara, Division of Communicable and Venereal Diseases, Department of Public Health, 600 Washington Street, Boston, Massachusetts 02111, USA

Accepted for publication 21 December 1981

Comment

Vaginal vibrators are of three types: one is stationary, another moves backwards and forwards like a piston, and a third (the type our patient used) has a rubberised "penis", which moves slowly in a circular motion (fig 2). All are battery or electrically operated and are provided with a lubricant.

Although not new, vaginal vibrators add one more aetiological agent to the differential diagnosis of cervical ulceration.

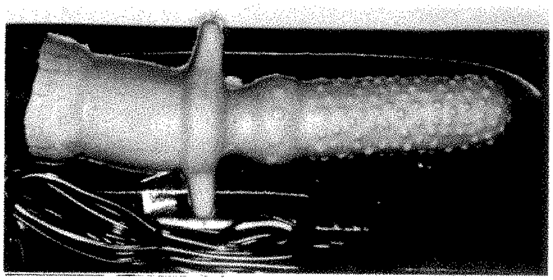


FIG 2 *A vaginal vibrator*

Reference

1. Willcox RR. Sexual behaviour and sexually transmitted disease patterns in male homosexuals. *Br J Vener Dis* 1981;**57**:167-9.

Br J Vener Dis 1982;**58:**204-5

World and Latin American congresses on sexually transmitted diseases

Report of the First World Congress and Third Latin American Congress on sexually transmitted diseases, San Juan, Puerto Rico, 15-21 November 1981

The First World Congress and the Third Latin American Congress on sexually transmitted diseases were held in San Juan, Puerto Rico, from 15 to 21 November 1981. More than 1400 participants from 50 different countries took part; they and their families and friends filled three resort hotels in Isla Verde.

The idea of the congress had originally been put forward by Dr Yamil H Kouri (Puerto Rico), and an organising committee headed by Dr Ronald K St John of the Pan American Health Organisation had been established the previous year. The congress was sponsored by the World Health Organisation, the International Union against the Venereal Diseases and Treponematoses, the Center for Disease Control, the National Institute of Health, the Latin American Union against the Venereal Diseases, and many other national and international agencies.

In his opening address the director of the congress, Dr Yamil Kouri, reminded delegates that the incidence of all the sexually transmitted diseases was increasing throughout the world and the number of diseases that were now known to be spread by this route was much greater than was generally believed. The fact that more than 1400 doctors from over 50 different countries, representing millions of patients all over the world, had come to the congress was an indication of the growing importance of the subject in modern medicine.

The members of the congress were welcomed by Dr N Maldonado, the chancellor of the University of Puerto Rico, the Hon Carlos Romera Barcelo, governor of the Commonwealth of Puerto Rico, Dr J Rivera Dueno, secretary to the Department of Health of the Commonwealth of Puerto Rico, Dr R D Catterall, president of the International Union against the Venereal Diseases and Treponematoses, Dr H Acuna, director of the Pan American Health Organisation, Dr W Foege, director of the Center for Disease Control, and the Hon R Schweiker, secretary of the Department of Health and Human Services of the Commonwealth of Puerto Rico.

Throughout the four principal days of the congress a number of sessions occurred simultaneously, and covered a wide variety of subjects. The programme contained a chart of all the sessions indicating in which of six possible locations a particular paper was to be presented. This arrangement allowed a large number of papers to be read but it also meant that important papers were often presented at the same time in different locations making it impossible to hear more than a selection of the communications. Some delegates found the programme too complicated and could not find the location at which specific subjects were to be discussed and they became confused and disorientated.

Nevertheless, the level of organisation was very high by international standards and most sessions took place without any difficulties. The speakers usually observed their time limits and the moderators conducted the discussions with firmness and discretion. There were, however, recurrent problems with the loud-speaker systems and with the slide projection services, a defect which is all too familiar and too common at medical and scientific meetings. Although the official language of the congress was English, simultaneous translation services were available at some of the sessions.

The first day was characterised by an excellent research symposium on the pathogenic mechanisms of bacterial sexually transmitted diseases. Dr P F Sparling spoke on gonorrhoea, Dr J Schachter on chlamydia, Dr R Swenson on group B streptococci, and Dr D Musher on syphilis. These papers set the standards for the whole conference and were listened to by a packed and appreciative audience. Later that day an excellent symposium on herpes simplex virus and cytomegalovirus provoked a lively discussion. Meanwhile, in other locations the efficacy of penicillin treatment of early syphilis in the prevention of neurosyphilis was discussed by Dr Axel Perdrup (Denmark), hepatitis B virus infections in venereal disease clinic patients by Dr P E Taylor (Canada), and infections caused by penicillinase-producing *Neisseria gonorrhoeae* in the United States from 1976 to 1981 by Dr H Joffe (United States).

Throughout the congress a series of STD update workshops, stressing the fundamentals of diagnosis and treatment, were held in several locations; poster sessions were established and there were panel discussions on a variety of subjects, such as herpes, children and STD, legal aspects of women and STD, and prostitution. The STD update programmes were of a very high standard and provided an excellent factual background to the research papers which were being presented simultaneously elsewhere.

A whole morning session on chlamydia and ureaplasmas provoked much discussion; simultaneously there was a session on methods of training for those working with STDs. The principles, methods, and current training practices in Britain, the USA, Latin America, and Africa were described and a variety of short-term training programmes mostly being developed in the USA were discussed. The methods employed in establishing a model STD clinic were described by Dr Yamil Kouri (Puerto Rico).

On the third day of the congress a morning session was devoted to the pathogenic mechanisms of viral STD, which included up-to-date papers on herpes simplex, cytomegalovirus, hepatitis B, and recent developments in the treatment of viral diseases. The session devoted to gonorrhoea was dominated by papers on resistance to penicillin and β-lactamase-producing strains of *Neisseria gonorrhoeae*. The session devoted to vaginal chemoprophylaxis in the prevention of STD was very disappointing as there were few new factual data, a dearth of current research, and an absence of new ideas. Many excellent poster displays, laboratory demonstrations, and audiovisual exhibits were available for

Address for reprints: Dr R D Catterall, James Pringle House, Middlesex Hospital Medical School, London W1A 8AA

Accepted for publication 17 January 1982

those who could find them in the maze of rooms in the three congress hotels.

On the fourth day a morning was devoted simultaneously to syphilis and to a general session on STD education. Much interesting research on *Treponema pallidum* was reported, and for those more interested in education, the objectives, evaluation, and practice of public health education on STDs were discussed at length.

On the last day a major research symposium on STD vaccines, antigenic determinants, and immune responses was attended by a packed auditorium. The situation concerning gonorrhoea was reviewed by Dr E Tramont (USA) and group B streptococci by Dr D Kasper. Owing to the illness of Dr W Szmuness his paper on hepatitis B was presented by Dr P E Taylor (Canada). An excellent review of other potential STD vaccines was given by Dr G Schoolnick (USA), and the session ended with a thought-provoking discussion paper on "Perspectives for public health usage of STD vaccines" by Dr K K Holmes (USA).

The formal sessions ended with a special update paper by Dr James Curran (USA) on Kaposi's sarcoma and the reports that the condition had been diagnosed in a number of homosexual men. The cause is unknown and the condition has been fatal in some of the patients.

It was fitting that the Parran Award for outstanding work on venereal diseases was given to Dr James Curran by the president of the American Venereal Diseases Association, and a number of awards were made by the Latin American Union against the Venereal Diseases.

The congress was finally closed with some concluding remarks by the chairmen of the various sections of the executive committee. Dr R St John (USA) announced that the executive committee was now dissolved but a recommendation had been made that a second world congress should be organised in about four to five years' time in view of the great success of the present congress.

Dr R D Catterall (London) thanked all those who had been concerned with the organisation of the congress and had contributed to the great success of the meeting and in particular Dr R St John (USA), who had been the main organiser of the whole congress and was responsible for the detailed planning and smooth running of all the sessions. But the originator of the idea and the man who had really made it all possible was Dr Yamil Kouri (Puerto Rico). Dr Catterall presented Dr Kouri with an engraved wall plaque, commemorating the congress, on behalf of all the delegates and participants and congratulated him on the very great success of the first world congress on sexually transmitted diseases.

During the course of the congress the International Union against the Venereal Diseases and Treponematoses held two executive committee meetings, which were both well attended. The American Venereal Diseases Association also held a meeting.

There was an excellent social programme during the day time; a reception-cocktail party, an evening Rum Swizzle party, a banquet, and big band dance were all highly successful and well attended.

It was generally agreed that the congress had been an unqualified success and had contributed greatly to the understanding of the many problems associated with the sexually transmitted diseases. It had also acted as a stimulus to all those working in the field and should be a reminder to politicians and health care administrators that this problem will require increased resources both now and in the future.

R D Catterall
(president, IUVDT)

Book review

Sexually Transmitted Diseases. By Mohamed Farid, 1981. Ain Shams University Press, Cairo, Egypt. Pp 647. Price not stated.

The author of this large volume is assistant professor of dermatology and venereology. His book has been published by his own University Press in Cairo but nevertheless appears to have been written in the United Arab Emirates, where he is also head of the department of dermatovenereology at the Military Hospital, Abu Dhabi. He has achieved a clear and comprehensive presentation of the sexually transmitted diseases, their problems and management, expressing views which are generally accepted in western countries. This is not surprising as it reflects well the information in the 175 or so world references quoted. In some 30 instances, however, passages or even whole paragraphs, with scant acknowledgement, have been taken verbatim or virtually so from *Sexually Transmitted Diseases* (C B S Schofield, 3rd ed, Edinburgh: Churchill Livingstone, 1979). Nevertheless, it could be a very useful vehicle to convey current trends in sexually transmitted diseases to physicians in the Middle East.

The chapters are conventionally arranged and cover chlamydial and the various viral infections, with a closing appendix on techniques and normal values of body fluids. There is also a chapter on the mechanism of erection and ejaculation, which devotes some space to priapism and its treatment but curiously makes no mention of impotence. Two short historical sections on syphilis and gonorrhoea list many famous people who are stated to have had these diseases.

The printing style is that of a double-spaced typewriter with only about half the usual number of lines per page, which results in a larger volume than usual. There are eight pages of colour pictures and a few diagrams. With the reservations already expressed there is much good sense in its contents, which read well with a commendable lack of embellishment. There are, however, some 60 noted errata (nearly all of spelling), some of which might have passed unnoticed had a slip detailing them not been supplied with the book.

R R Willcox

Br J Vener Dis 1982;**58**:206-10

Abstracts

These selected abstracts and titles from the world literature are arranged in the following sections:

Syphilis and other treponematoses
(Clinical and therapy; serology and biological false-positive phenomenon; pathology and experimental)
Gonorrhoea
(Clinical; microbiology; therapy)
Non-specific genital infection
Reiter's disease
Trichomoniasis
Candidosis
Genital herpes
Other sexually transmitted diseases
Public health and social aspects
Miscellaneous

Syphilis and other treponematoses (clinical and therapy)

A case of monorecidive syphilitic chancre
SP MEHTA (Special Clinic, Royal Hospital, Wolverhampton, UK) *Sex Transm Dis* 1981;**8**:222-3.

Tabetic lightning pains: High-dosage intravenous penicillin versus carbamazepine therapy
S GIMENÉZ-ROLDÁN AND M MARTIN (Ciudad Sanitaria Provincial, Madrid, Spain) *Eur Neurol* 1981;**20**:424.

Syphilis (serology and biological false-positive phenomenon)

Specificity, sensitivity and reproducibility among the fluorescent treponemal antibody-absorption test, the microhaemagglutination assay for *Treponema pallidum* antibodies, and the haemagglutination treponemal test for syphilis
SA LARSEN, EA HAMBLE, DE PETTIT, *ET AL* (Center for Disease Control, Atlanta, GA, USA). *J Clin Microbiol* 1981;**14**:441-5.

Serodiagnosis of syphilis (lues)
F MULLER (Hamburg, Germany). *Bakt Mikrobiol Hyg-A-Med* 1981;**250**:1-8.

Syphilis (pathology and experimental)

Isolation of a heat stable antigen from *Treponema* Reiter using an immunoadsorbent with antibodies from syphilitic patients
NS PEDERSON, CS PETERSEN, NH AXELSEN, *ET AL* (Statens Seruminstitut, Copenhagen, Denmark). *Scand J Immunol* 1981;**14**:137-44.

In-situ identification of mononuclear cells in cutaneous infiltrates in discoid lupus erythematosus, sarcoidosis, and secondary syphilis
JR BJERKE, H-K KROGH, AND R MATRE (University of Bergen, Norway). *Acta Derm Venereol* 1981;**61**:371-80.

Pathogenesis and immunology of *Treponema pallidum*
TJ FITZGERALD (University of Minnesota, Duluth, Minnesota, USA). *Ann Rev Microbiol* 1981;**35**:29-54.

Effect of cortisone administration on host-parasite relationships in early experimental syphilis
SA LUKEHART, SA BAKER-ZANDER, RM CHERI LLOYD, AND S SELL (Department of Pathology, School of Medicine, University of California, San Diego, La Jolla, CA, USA). *J Immunol* 1981;**127**:1361-8.

Experiments were carried out to investigate the action of cortisone acetate on experimental syphilis in rabbit testes during the three phases of orchitis development: (a) before lymphocyte sensitisation and cellular infiltration; (b) after lymphocyte sensitisation but before bacterial clearance; and (c) after bacterial clearance from the site of infection. Four of six groups of New Zealand white rabbits were each infected intratesticularly with 8 × 10^7 *T pallidum*/testis. The first two groups (A and B) were used as controls: A received no steroids and B were given cortisone acetate 20 mg/day intramuscularly. The infected groups (D, E, and F) were given cortisone acetate on days 0-6, 7-13, and 21-27 respectively. Group C, although infected, had no steroids and acted as another control group.

Rabbits were killed at weekly intervals, and the spleen, testes, inguinal and popliteal lymph nodes, and blood were all examined. The sera were tested by the VDRL and FTA-ABS tests. The spleen and lymph nodes were both used for lymphocyte stimulation tests and the testes sectioned for immunofluorescent staining.

The results showed that cortisone suppressed the VDRL titres but that the titres regained the values of group C rabbits within three days (group D) or one week (groups E and F). The FTA-ABS test showed a delay in antibody development in group D, a small drop in titres in group E between days 14 and 21, and no apparent effect from steroids in group F. The lymphocyte stimulation tests with *T pallidum* antigen showed a delay in appearance of sensitised spleen cells in group D, an ablation in response in group E, and a reduction in sensitised cells in group F. In each group responsiveness recurred seven days after treatment, although in group D it took longer to return completely to the values of group C. Histological examination of the testes in group C supported the findings previously reported by the same authors.[1] They used the same double antibody technique to detect *T pallidum*, T lymphocytes, and IgG in sections of the testes from the rabbits in groups D, E, and F. Group D rabbits showed T lymphocytes on day 10, which had diminished in number on day 14. Treponemes were present on day 7 and day 14. In group E the local inflammatory response was present at the time of the first cortisone injection; it was noticeably less at the end of the course, with many fewer lymphocytes than in group C. Treponemes were found on day 17 and were still present on day 28, by which time the group C animals showed very few treponemes at all.

Foamy macrophages stained with haematoxylin and eosin also gave a strongly positive immunofluorescent response for *T pallidum*, implying ingestion of the organism as a means of clearance from the site of infection. Their appearance was delayed by the cortisone administration. Group F animals were given cortisone when the local inflammatory response was diminished with very few treponemes present in the testes. Immediately after the cortisone course (day 28) focal accumulations of *T pallidum* could be found. Fibroblasts which had previously been present began to loosen by day 35, when an increase in T lymphocytes was noted together with larger numbers of treponemes.

The authors discuss their findings with reference to earlier work on the subject. As in their previous papers, the photomicrographs are of very good quality.

1. Lukehart SA, Baker-Zander SA, Cheri Lloyd RM, Sell S. *J Immunol* 1980;**124**:461-7.

G D Morrison

Gonorrhoea (clinical)

Gonococcal carriage in the throat; no common findings anyhow? Absence of *Neisseria gonorrhoeae* in the pharynges of 158 repeatedly swabbed young men

HC KORTING (Ernst Rodenwaldt Institute, Koblenz, Federal German Republic). *Dermatologica* 1981;**163**:249-54.

Gonorrhoea (microbiology)

Application of a limulus test device in rapid evaluation of gonococcal and non-gonococcal urethritis in males

RB PRIOR AND VA SPAGNA (Ohio State University College of Medicine, Columbus, USA). *J Clin Microbiol* 1981; **14**:256-60.

The limulus amoebocyte lysate (LAL) assay depends on the principle that washed amoebocytes of the horseshoe crab, *Limulus polyphemus*, will form a gel in the presence of nanogram quantities of bacterial endotoxin. Endotoxin in urethral exudates from men with gonococcal urethritis can also give a positive LAL assay result. This paper evaluates a test device developed to simplify and standardise the collection, dilution, and testing of urethral exudates. The test device consists of a syringe for collecting specimens, a sealed dilution reservoir containing 10 ml pyrogen-free water, and a lysate single-test vial. The dilution reservoir contains a frangible membrane, which is broken before use. The sample is collected from the urethral meatus by gentle aspiration until the exudate fills the syringe-tip from one half to full (approximately 0·015 to 0·025 ml). The syringe is then affixed to the dilution reservoir and the sample expressed into the reservoir. After adequate mixing 0·25 ml of the diluted sample is removed and transferred to the assay vial with the collecting syringe. The vial is incubated at 37°C for 30 minutes before the test result is read.

Five hundred and fifty men with uncomplicated exudative urethritis were included in the evaluation of the device. After exudate for the assay had been collected, samples were also collected for Gram staining and culture of *Neisseria gonorrhoeae*. Of the 550 men, 366 had positive culture results for *N gonorrhoeae* and 184 negative results. On the basis of culture results the sensitivity with the LAL device was 99·2% and the specificity 96·7%: the corresponding values with Gram stain were 96·4% and 99·5%. There were no statistically significant differences between the LAL assay and the Gram stain in predicting culture result ($P>0·05$). The value of the device is discussed in relation to the practising physician in the private sector in the United States. (Since Gram-staining of urethral exudate gives rapid reliable results and is inexpensive, there is little need for the LAL device in the presumptive diagnosis of gonococcal urethritis in men attending STD clinics in Britain.).

H Young

Evaluation of the limulus amoebocyte lysate assay for the presumptive diagnosis of gonorrhoea in men at a clinic for sexually transmitted diseases

TA CHAPEL, M ADCOCK, B SMITH, *ET AL* (Wayne State University School of Medicine, Detroit, USA). *Sex Transm Dis* 1981;**8**:175-8.

Gonococcal pilus vaccine—studies of antigenicity and inhibition of attachment

EC TRAMONT, JC SADOFF, JW BOSLEGO, *ET AL* (Walter Reed Army Institute, Washington DC, USA). *J Clin Invest* 1981;**68**:881-9.

Antibiotic susceptibility of *Neisseria gonorrhoeae* in relation to serogroups

S BYGDEMAN (Karolinska Institute, Stockholm, Sweden). *Acta Pathol Microbiol Scand* 1981;**89**:227-38.

Antibiotic susceptibility testing of *Neisseria gonorrhoeae* by disc agar diffusion

JY RIOU, M GUIBOURDENCHE, AND P COURVALIN (Institute Pasteur, Paris, France). *Ann Microbiol* 1981;**132**:41-50.

Survey of *Neisseria gonorrhoeae* sensitivity to antibiotics by dilution and diffusion methods using a new medium

E DELBEKE, J CHAMBON, D SICARD, *ET AL* (Faculty of Medicine and Chemistry, Vallombrose, Nice, France). *Ann Microbiol* 1981;**132**:51-7.

Some properties of the human erythrocyte receptors for *Neisseria gonorrhoeae*

GM WISEMAN, P McNICOL, CJ LIAN, AND DS PRIMROSE (University of Manitoba School of Medicine, Winnepeg, Canada). *Can J Microbiol* 1981;**27**:1035-47.

Degradation of gonococcal outer membrane proteins by human neutrophil lysosomal proteases

RF REST AND E PRETZER (Arizona Health Science Center, University of Arizona, Tucson, AZ, USA). *Infect Immun* 1981; **34**:62-8.

Enzyme linked immunosorbent assay (ELISA) to detect antibodies in gonorrhoea using whole cells

CA ISON, SG HADFIELD, AND AA GLYNN (Central Public Health Laboratory, Colindale, London, UK). *J Clin Pathol* 1981;**34**:1040-3.

Intraspecific and intergeneric mobilisation of non-conjugative resistance plasmids by a 24·5 megadalton conjugative plasmid of *Neisseria gonorrhoeae*

F FLETT, GO HUMPHREYS, AND JR SAUNDERS (Department of Biochemistry, University of Manchester Institute of Science and Technology, Manchester, UK). *J Gen Microbiol* 1981; **125**: 123-30.

Serological classification of *Neisseria gonorrhoeae* by coagglutination: a study of serological patterns in two geographical areas of Sweden

S BYGDEMAN, D DANIELSSON, AND E SANDSTRÖM (Department of Clinical Bacteriology, Sodensjukhuset, Stockholm, Sweden). *Acta Derm Venereol* 1981; **61**: 423-8.

Gonorrhoea (therapy)

Treatment of uncomplicated gonorrhoea with cefotaxime

HH HANDSFIELD AND KK HOLMES (University of Washington, Harborview Medical Center, Seattle, USA). *Sex Transm Dis* 1981; **8**: 187-91.

Cefuroxime therapy of gonorrhoea and coinfection with *Chlamydia trachomatis* in children

P PATAMASUCON, PJ RETTIG, AND JD NELSON (University of Texas Health Science Center, Dallas, Texas, USA). *Pediatrics* 1981; **68**: 534-8.

Twenty-seven episodes of gonorrhoea occurring in children under 15 years of age were treated with intramuscular injections of cefuroxime 25 mg/kg body weight. Gonococci were eliminated from genital, pharyngeal, and anal sites in all cases. Cefuroxime was well tolerated and no side effects were observed. Concomitant infection with *Chlamydia trachomatis* was found in nine (33%) patients. Of the seven patients with chlamydial genital infection, postgonococcal symptoms persisted in three (43%). One of the five patients with chlamydial anal infection had symptoms. Chlamydial infections were successfully treated with trisulfapyrimidines.

Authors' summary

Non-specific genital infection

Incidence of *Ureaplasma urealyticum* infection in women attending a clinic for sexually transmitted diseases

H YOUNG, S TUACH, AND SSR BAIN (Department of Bacteriology, University of Edinburgh Medical School, Edinburgh, UK). *J Infect* 1981; **3**: 258-65.

Isolation of *Chlamydia trachomatis* from women with urethral syndrome

A WEIL, R GAUDENZ, L BURGENER, AND B SCHULTZ (University of Geneva, Geneva, Switzerland). *Arch Gynaecol* 1981; **230**: 329-34.

Cellular immune response during uncomplicated genital infection with *Chlamydia trachomatis* in humans

RC BRUNHAM, DH MARTIN, CC KUO, *ET AL* (United States Public Health Service Hospital, Seattle, WA, USA). *Infect Immun* 1981; **34**: 98-104.

Cystitis associated with chlamydial infection of the genital tract in male guinea pigs

RG RANK, HJ WHITE, BL SOCOFF, AND AL BARRON (Department of Microbiology and Immunology, University of Kansas, Little Rock, Arkansas, USA). *Sex Transm Dis* 1981; **8**: 203-10.

In-vitro activity of clindamycin against *Chlamydia trachomatis*

WR BOWIE (Vancouver General Hospital, Vancouver, BC, Canada). *Sex Transm Dis* 1981; **8**: 220-2.

The prevalence of *Ureaplasma urealyticum* and *Mycoplasma hominis* in the cervix and anal canal of women

PE MUNDAY, PM FURR, AND D TAYLOR-ROBINSON (Clinical Research Centre, Harrow, Middlesex, UK). *J Infect* 1981; **3**: 253-7.

Isolation of *Chlamydia trachomatis* from the urethra and from the prostatic fluid in men with signs and symptoms of acute urethritis

S NILSSON, G JOHANNISSON, AND E LYCKE (Department of Urology, Gothenburg University, Gothenburg, Sweden). *Acta Derm Venereol* 1981; **61**: 456-8.

Sclerosing lymphangitis of the penis: a possible chlamydial aetiology

JK KRISTENSEN AND J SCHEIBEL (University Institute of Biology and Chemistry, Copenhagen, Denmark). *Acta Derm Venereol* 1981; **61**: 455.

Infection with *Chlamydia trachomatis, Mycoplasma hominis,* and *Neisseria gonorrhoeae* in patients with acute pelvic inflammatory disease

BR MØLLER, P-A MÅRDH, S AHRONS, AND E NÜSSLER (Aarhus University, Aarhus, Denmark). *Sex Transm Dis* 1981; **8**: 198-202.

Acute salpingitis with *Chlamydia trachomatis* isolated from fallopian tubes: clinical, cultural, and serologic findings

L SWENSSON, L WESTRÖM, AND P-A MÅRDH (University of Lund, Sweden). *Sex Transm Dis* 1981; **8**: 51-5.

Ten patients, from whose fallopian tubes *Chlamydia trachomatis* was isolated at laparoscopy, were assessed for clinical symptoms and signs and microbiological findings. Five patients presented with irregular bleeding, nine with increased vaginal discharge, and two with urinary symptoms. The onset of pelvic pain varied from insidious to more acute with a duration ranging from three to 27 days. It had been thought that chlamydia-associated salpingitis could run a relatively benign course. One patient aged 18 years (median age of group, 19 years) had a rectal temperature of more than 38°C.

Although two patients complained of only brief pelvic pain (four days' duration), severe inflammatory changes were present on laparoscopy with pelvic peritonitis or abscess formation with closed ostia or both. A further five patients had moderate inflammation with adhesions and ESR ranging between 10 to 90 mm/first hour. The remaining three patients had only mild inflammation. The four patients using intrauterine contraceptive devices did not have more pronounced signs or symptoms than three others using oral contraception.

Although *C trachomatis* was not isolated from the cervix in two patients, it was still grown from the fallopian tubes. Serological tests showed a rise in chlamydial IgG antibody titres in six out of eight patients. IgM antibodies were found in only two patients; previous chlamydial urogenital infections probably accounted for this sparsity.

Cervical *Mycoplasma hominis* was detected in one patient. In another, stationary antibody titres (IHA) were noted, but four patients examined for *Mycoplasma hominis* in the fallopian tubes had negative results. *Bacteroides* species were also excluded when samples were taken from either the tubes or the utero-rectal pouch.

Two patients had positive culture results for *N gonorrhoeae* from the cervix with accompanying antibodies (IHA) to gonococcal pili. These patients were admitted after three days of pelvic pain and had negative results for *N gonorrhoeae* from the fallopian tubes. One of these patients had stationary antibody titres to *C trachomatis* and mild inflammation on laparoscopy but nevertheless harboured chlamydia in the tubes. The authors have therefore shown that in clinical practice this finding, together with a negative cervical culture result for *C trachomatis*, is not incompatible with an underlying salpingitis of chlamydial origin.

J M Harvey

Trichomoniasis

Metronidazole metabolism in cultures of *Entamoeba histolytica* and *Trichomonas vaginalis*

BB BEAULIEU, JRMA McLAFFERTY, RL KOCH, AND P GOLDMAN (Harvard University School of Medicine, Boston, Massachusetts, USA). *Antimicrob Agents Chemother* 1981;**20:**410-4.

It has long been known that metronidazole is not itself active but that after being taken up by the target organism it is reduced to an active metabolite which actually causes cell death. This metabolite itself is too labile to be isolated and characterised, but plausible structures for it suggest that it should react with water to give a stable and detectable product acetamide. In this paper the authors, having previously shown that acetamide is produced by the incubation of anaerobic bacteria with metronidazole, demonstrated that 2 ^{14}C metronidazole added to cultures of *Entamoeba histolytica* led to the formation of ^{14}C-labelled acetamide. They then incubated two strains of *Trichomonas vaginalis* (one, IR78, metronidazole-resistant) under aerobic conditions with ^{14}C-labelled metronidazole and showed that the drug-resistant strain produced rather less acetamide than the sensitive strain. No quantitative studies were possible when the experiments were repeated under anaerobic conditions (when both strains are sensitive to metronidazole), but both strains seemed to produce about the same (2-4 times larger) quantities of acetamide.

Although the figures in this paper do not show dramatic differences they are at least compatible with the hypothesis that metronidazole resistance in *T vaginalis* is accompanied by reduced production of the active metabolite.

J P Ackers

In-vitro sensitivity of *Trichomonas vaginalis* to zinc

WA GARDNER Jr, CE O'HARA, J BAILEY, AND BD BENNET (University of Southern Alabama, Mobile, Alabama, USA). *Prostate* 1981;**2:**323.

Activation of the alternative complement pathway of *Trichomonas vaginalis*

FD GILLIN AND A SHER (Parasitic Disease Laboratory, NIAID, Bethesda MD, USA). *Infect Immun* 1981;**34:**268-73.

Candidosis

A corticosteroid binding protein and endogenous ligand in *C albicans* indicating a possible steroid receptor system

DL LOOSE, DJ SCHURMAN, AND D FELDMAN (Stanford University Medical Center, Stanford, California, USA). *Nature* 1981; **293:**477-8.

Precipitation tests in candidiasis

PG SHIVANANDA, JN SARVAMANGALA, AND KNA RAO (Kasturba Medical College, Manipal, India). *Ind J Med Res* 1981;**74:** 358-64.

Treatment of vulvovaginal candidiasis with boric acid powder

KK VAN SLYKE, VP MICHEL, AND MF REIN (University of Virginia, School of Medicine, Charlottesville, Virginia, USA). *Am J Obstet Gynecol* 1981;**141:**145-8.

A double-blind comparison was made of the use of 14 daily intravaginal gelatin capsules containing 600 mg of boric acid powder versus the use of identical capsules containing 100 000 U nystatin diluted to volume with cornstarch for the treatment of vulvovaginal candidiasis. Cure rates for boric acid were 92% at 7-10 days after treatment and 72% at 30 days, whereas the nystatin cure rates were 64% at 7-10 days and 50% at 30 days. The speed of alleviation of signs and symptoms was similar with the two drugs. There were no untoward side effects, and cervical cytological features were not affected. In-vitro studies found boric acid to be fungistatic and its effectiveness to be unrelated to pH. Blood boron analyses indicated little absorption from the vagina and a half-life of less than 12 hours. Acceptance by the patients was better than for 'messy' vaginal creams, and self-made capsules containing boric acid powder are inexpensive compared with the costly medication commonly prescribed.

Authors' summary

Genital herpes

Induction of uterine cancer with inactivated herpes simplex virus types 1 and 2

WB WENTZ, JW REAGAN, AD HEGGIE, *ET AL* (Case Western Reserve University, Cleveland, Ohio, USA). *Cancer* 1981;**48:** 1783-90.

Other sexually transmitted diseases

The etiology of anorectal infections in homosexual men

TC QUINN, L COREY, RG CHAFFEE, *ET AL* (University of Washington, Seattle, USA). *Am J Med* 1981;**71:**395-406.

A study was carried out of 52 homosexual men over a period of 42 months who complained of symptomatic anorectal disease and gave no recent history of having been contacts of men with known urethral gonorrhoea. Investigations included a Gram stain of rectal exudate (not mentioned if taken by direct proctoscopy), urethral, pharyngeal, and rectal cultures for *Neisseria gonorrhoeae*, rectal cultures for herpes simplex virus (HSV), serological tests for syphilis (VDRL), and in certain cases stool cultures for *Salmonella, Shigella, Chlamydia trachomatis,* and *Campylobacter foetus* ssp, and stool examination for ova and parasites. In the latter part of the study sigmoidoscopy was performed on 22 men, rectal biopsy being performed if abnormal findings were present. Biopsy specimens were cultured for HSV, *N gonorrhoeae, C trachomatis,* and bacterial enteric pathogens and a specimen obtained for examination for ova and parasites. Biopsy specimens were also sent for histology.

The men's ages ranged from 18-41 years (mean 26·4 years); 90% were white; and all give a history of anilingus, fellatio, or anoreceptive homosexual intercourse. The number of sexual partners ranged from one to 11 (mean 6·1) a month. Their symptoms were as follows: anal discharge, 89%; rectal pain, 87%; diarrhoea, 48%; constipation, 42%; bloody stools, 37%; tenesmus, 35%; abdominal pain, 35%; fever, 27%; and pruritus ani, 10%.

HSV was found in 15 (29%) of 52 men, the majority complaining of ano-rectal pain; focal ulceration was present. Despite negative results for Gram staining seven (14%) had rectal gonorrhoea; six (12%) had syphilis, two having anal specimens which gave positive results by darkfield microscopy. Four harboured enteric pathogens: two *Entamoeba histolytica,* two *Giardia lamblia,* and one *Campylobacter foetus* ssp *jejuni* (concomitant enteric pathogen infection occurred in the group). *C trachomatis* (LGV-2 strain) was isolated from one patient with severe granulomatous proctitis. Six (12%) patients had anal condylomata acuminata. One or more pathogens were identified in 28 (67%) of 42 men who had anorectal leucocytic exudate and in two of 10 who did not. In 13 (52%) of 25 in the first part of the study and in nine (33%) of 27 in the latter part, no pathogens were found.

Of the remaining 27 patients, 22 underwent sigmoidoscopy; 10 had sigmoidoscopic or histological evidence, or both, of acute proctitis, and eight of the remaining 12 had polymorphonuclear leucocytes in rectal specimens. An infectious pathogen was found in 13 (72%) of 18 with proctitis or anorectal leucocytes or both and in none of four with apparently normal mucosa and no leucocytes.

The remainder of the study reviews recent literature on anorectal gonorrhoea, HSV infection, syphilis, and enteric pathogen infections together with case studies illustrating features, anorectal chlamydial infection, and other causes of anorectal disease in homosexuals. (The bibliography is one of the most thorough yet on recent work on homosexually transmitted infections).

Michael Waugh

Treatment of chancroid

JE FITZPATRICK, H TYLER, AND ND GRAMSTAD (Fitzsimons Army Medical Center, Aurora Co, USA). *JAMA* 1981;**246:**1804-5.

Thirty-five men with chancroid were randomly treated with oral sulphisoxazole, sulphisoxazole and tetracycline, sulphamethoxazole-trimethoprim, or intramuscular streptomycin. The highest cure rates were obtained in 13 of 13 patients treated with streptomycin and in 10 of 10 patients treated with sulphamethoxazole-trimethoprim. Only seven of nine patients treated with sulphisoxazole and five of eight treated with sulphisoxazole and tetracycline were cured. The authors conclude that the sulphamethoxazole-trimethoprim combination is as efficacious as streptomycin and probably superior to sulphisoxazole and tetracycline in the treatment of chancroid.

Authors' summary

Malignant transformation of perianal condylomata acuminata. A case report with review of the literature

SH LEE, DH McGREGOR, AND MN KUZIEZ (Veterans Administration Medical Center, Kansas City, USA). *Dis Colon Rectum* 1981;**24:**462-7.

Penile condylomata acuminata: an experimental model for evaluation of topical self treatment with 0·5%-1·0% ethanolic preparations of podophyllotoxin for three days

G von KROGH (Sodersjukhuset, S-10064 Stockholm, Sweden). *Sex Transm Dis* 1981;**8:**179-86.

Treatment of chancroid with erythromycin

JL CARPENTER, A BACK, D GEHLE AND T OBERHOFFER (Brodie Army Medical Center, Houston, Texas, USA). *Sex Transm Dis* 1981;**8:**192-7.

Molluscum contagiosum

ST BROWN, JF NALLEY, AND SJ KRAUS (Center for Disease Control, Atlanta, Georgia, USA). *Sex Transm Dis* 1981;**8:**227-34.

Infant pneumonitis associated with cytomegalovirus, chlamydia, pneumocystis, and ureaplasma: a prospective study

S STAGNO, DM BRASFIELD, MB BROWN, *ET AL* (Department of Pediatrics, University of Alabama, Birmingham, Alabama, USA). *Pediatrics* 1981;**68:**322-9.

Public health and social aspects

Infections due to penicillinase-producing *Neisseria gonorrhoeae* in the United States 1976-1980

HW JAFFE, JW BIDDLE, SR JOHNSON, AND P WIESNER (Center for Disease Control, Atlanta, Georgia, USA). *J Infect Dis* 1981;**144:**191-7.

Penicillinase-producing *Neisseria gonorrhoeae* in Canada

JR DILLON, M PAUZE, AND AG JESSAMINE (Laboratory Center for Disease Control, Ottawa, Canada). *Can Med Assoc J* 1981; **125:**851-6.

M.S.S.V.D.

The Medical Society for the Study of Venereal Diseases

11 Chandos Street, Cavendish Square, London W1M 0EB

President: Dr. Jack Barrow

The object of the Society is to bring together those interested or engaged in the diagnosis, treatment, and public health aspects of venereal diseases. The Society meets regularly in London for the reading of papers, discussions, and presentation of cases. An out-of-London meeting is held in the Spring.

Annual subscription for membership of the Society is £20 for all members.

Applications for membership are very welcome and forms can be obtained from the Honorary Secretary, Dr. M. A. Waugh, Department of Genito-Urinary Medicine, The General Infirmary Great George Street, Leeds LS1 3EX.

Proposed Programme 1982 – 1983

Friday 29th October, 1982	1. Annual General Meeting. 2. Short papers.
Friday 26th November, 1982	Hepatitis B.
Friday 28th January, 1983	Prostatitis.
Friday 25th March, 1983	Clinical meeting to be arranged.
Friday 29th April, 1983	Presidential Address. Dr. Elizabeth Rees.
May. 13th – 15th, 1983	Spring Meeting, Rotterdam, the Netherlands.

The University of Liverpool

DIPLOMA IN VENEREOLOGY

The annual full-time three-months- course in venereal and sexually transmitted diseases, leading to the Diploma in Venereology, of the University of Liverpool, will commence in January, 1983.

The course covers the whole field of sexually transmitted infection and includes lectures and laboratory instruction.

Application forms are available from:

The Dept. of Venereology,
'Q' Clinic,
Royal Liverpool Hospital,
Prescot Street,
Liverpool L7 8XP.

and should be returned, duly completed by 30th June, 1982.

Drugs and Disease

The Proceedings of a Symposium
organized by the
Royal College of Pathologists

Edited by
Sheila Worlledge

Price: Inland £3·00
Abroad US $7·50
including postage

The Publishing Manager, JOURNAL OF CLINICAL PATHOLOGY, BMA House, Tavistock Square, London WC1H 9JR

Sexually Transmitted Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $30.00 outside the United States ($25.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $33.00 and $36.00 in the United States and $37.00 and $40.00 in all other countries.

William O. Harrison, M.D.
American Venereal Disease Association
P.O. Box 22349
San Diego, California 92122 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $30.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases*.

☐ I wish to enter a Non-Member or institutional subscription ($33.00 and $36.00 U.S., all other countries $37.00 and $40.00).
(Mail non-member or institutional subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105).

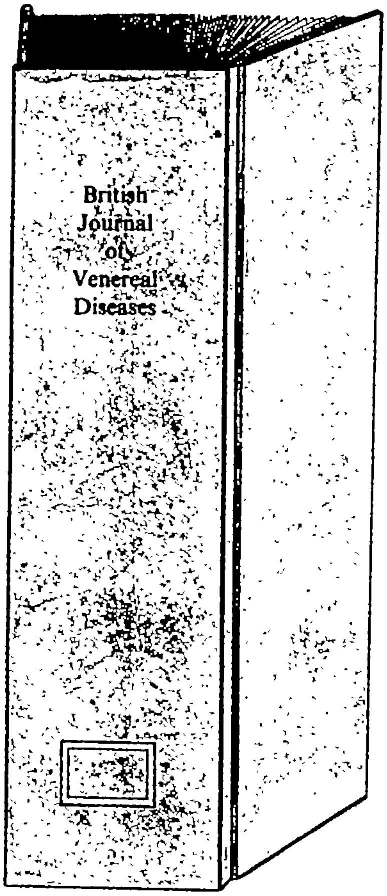
Name ____________________

____

Address ____________________

City ____________ State Country ____________ Zip ____________

Available January 1982



'CORDEX'

SELF-BINDING CASES

for easy and instant reference

Made to hold two volumes (12 issues) of your **BRITISH JOURNAL OF VENEREAL DISEASES.** Each case is handsomely bound with a colour to complement the journal. Spring nylon cords, to carry the journals, slide freely along the spine of the binder. The name of the journal is gold-blocked on the spine. Date labels are also provided.

Price: Inland £4.00; Overseas US$12.00
including postage (air mail overseas) and packing

ORDER NOW

From: The Subscription Manager,
BRITISH JOURNAL OF VENEREAL DISEASES
BMA House, Tavistock Square, London WC1H 9JR

British Journal of Venereal Diseases June 1982 Vol 58 No 3

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Effects of anaerobic and microaerophilic conditions of extraction and incubation on the survival of *Treponema pallidum* in vitro
G H W WONG, B M STEINER, AND S R GRAVES *page* 139

Interferon in rabbit sera after inoculation with *Treponema pallidum* suspensions contaminated with PED virus
K L FENNESTAD, L BRUUN, AND S HAAHR *page* 143

Evaluation of the role of the *Treponema pallidum* immobilisation test in Britain
M S SPROTT, J B SELKON, AND R H TURNER *page* 147

Syphilis d'emblée due to blood transfusion: case report
AGUS SOENDJOJO, MARIANI BOEDISANTOSO, MOCHAMAD I ILIAS, AND DIBJO RAHARDJO *page* 149

Morbidity associated with pelvic inflammatory disease
MICHAEL W ADLER, ELIZABETH H BELSEY, AND BRENDAN H O'CONNOR *page* 151

Neisseria gonorrhoeae isolated from disseminated and localised infections in pre-penicillin era: auxotypes and antibacterial drug resistances
B WESLEY CATLIN AND ALICE REYN *page* 158

Auxotypes and antibacterial resistance of gonococci with differing susceptibilities to vancomycin
A CHARLES EXNER, ELIZABETH NASH SHINNERS, PAUL J PACE, AND B WESLEY CATLIN *page* 166

Single oral dose of cefaclor for the treatment of infections with penicillinase-producing strains of *Neisseria gonorrhoeae*
THELMA E TUPASI, OFELIA V CALUBIRAN, AND CLEOTILDE A TORRES *page* 176

Treatment of uncomplicated gonorrhoea in women with talampicillin in a single oral 1·5 g dose
K C MOHANTY AND R B ROY *page* 180

Therapeutic abortion and *Chlamydia trachomatis* infection
ERIK QVIGSTAD, KJELL SKAUG, FRIDTJOF JERVE, INGER S S VIK, AND JAN C ULSTRUP *page* 182

Some factors affecting the incubation period and duration of symptoms of urethritis in men
C B S SCHOFIELD *page* 184

Ultrahistopathology of balanitis circinata
LASSE KANERVA, MERJA KOUSA, KIRSTI-MARIA NIEMI, ALLAN LASSUS, TIMO JUVAKOSKI, AND JORMA LAUHARANTA *page* 188

Rheumatoid arthritis, ankylosing spondylitis, and Reiter's syndrome occurring simultaneously: case report
H A LAVERY, T HORNER, AND S D ROBERTS *page* 196

Vaginal bleeding in granuloma inguinale: case reports
S MURUGAN, KAMALAKSHI VENKATRAM, AND P S RENGANATHAN *page* 200

Traumatic ulceration of the cervix: case report
NICHOLAS J FIUMARA AND THOMAS CAHN *page* 202

World and Latin American congresses on sexually transmitted diseases
R D CATTERALL *page* 204

Book review *page* 205

Abstracts *page* 206

ASTM CODEN: BJVDAK 58 (3) 139-210 (1982)
ISSN 0007-134X

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

December 1982 Vol. 58 No. 6

British Journal of
Venereal Diseases

BRITISH MEDICAL ASSOCIATION TAVISTOCK SQUARE LONDON WC1

British Journal of Venereal Diseases

A McMILLAN
(Editor)

B A EVANS
(Assistant Editor)

G D MORRISON
(Assistant Editor, Abstracts)

E REES
(President, MSSVD)

M WAUGH
(Hon Sec. MSSVD)

M W ADLER

G CAUSSE

G CSONKA

L M DRUSIN

R S PATTMAN

I PHILLIPS

E STOLZ

D TAYLOR-ROBINSON

R N THIN

J WALLIN

A E WILKINSON

EDITOR
British Medical Journal

P D SIMMONS
(Indexer)

SHUNA JUDGE
(Technical Editor)

This *Journal,* founded by the Medical Society for the Study of the Venereal Diseases, publishes original work on the investigation and treatment of venereal diseases, sexually transmitted diseases and allied disorders, and review articles, correspondence, and abstracts.

ADVICE TO AUTHORS Papers, which will be accepted on the understanding that they have not been and will not be published elsewhere and are subject to editorial revision, should be sent in duplicate to Dr A McMillan, Dept of Genitourinary Medicine, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW. The covering letter should contain a statement that the manuscript has been seen and approved by all authors. Any change of address of the corresponding author between submission and publication of the paper should be notified in advance to the Technical Editor, C/O BMA House. Manuscripts will only be acknowledged if a stamped addressed postcard or international reply coupon is enclosed.

Manuscripts must be typewritten on one side of the paper only in double-spacing with ample margins. Each manuscript should include (in that order): a brief summary outlining the main observations and conclusions; the text divided into appropriate sections; acknowledgements; references; tables, each on a separate sheet; and legends for illustrations: Only recognised abbreviations should be used. Full details of requirements for manuscripts (in accordance with the Vancouver agreement) are given in *Uniform Requirements for Manuscripts Submitted to Biomedical Journals,* available from the Publishing Manager, *British Medical Journal,* BMA House (50p post free).

ILLUSTRATIONS These should be marked on the back with the author's name and the top edge indicated. Photographs should be of the size desired for reproduction; photomicrographs must have internal scale markers. *X*-ray films should be submitted as photographic prints, carefully prepared so that they bring out the exact point to be illustrated. Diagrams should be submitted as unmounted, glossy, black-and-white prints where possible.

REFERENCES Authors must take full responsibility for the accuracy of their references and the list should be kept as short as practicable. References must be identified by arabic numerals in the text, tables, and legends. In the list of references they should be numbered in the consecutive order in which they first appeared in the text. The author's name and initials should be followed by the title of the paper and the name of the journal (abbreviated according to the style used in *Index Medicus*) together with the year of publication, volume number, and first and last page numbers. Where there are seven or more authors, only the names of the first three should be given followed by *et al.*

1. Thin RN, Shaw EJ. Diagnosis of gonorrhoea in women. *Br J Vener Dis* 1979; **55**: 224-5.

For books, the author's name and initials, full title, edition, place of publication, publisher, and year of publication should be given in that order.

2. King A, Nicol CS, Rodin P. *Venereal Diseases.* 4th ed. London: Baillière and Tindall, 1980.

Where the names of editors, compilers, or an agency are also given, or a chapter in a book is referred to, the reference should be quoted as follows:

3. Wentworth BB. Sensitivity of cell culture for isolation of *Chlamydia trachomatis* from genital sources. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 214-22.

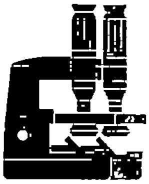
PROOFS Contributors will receive one proof, and should read it carefully for printers' errors, and check the tables, figures, legends, and any numerical, mathematical, or other scientific expressions. Alterations to the original text should be kept to a minimum.

REPRINTS 25 reprints will be supplied free of charge. A limited number of additional reprints may be ordered from the Publishing Manager when the proofs are returned.

NOTICE TO SUBSCRIBERS This Journal is published six times a year. The annual subscription rate including postage by surface mail is £31.00 in the United Kingdom and Republic of Ireland and US\$76.00 in all countries overseas. Payment for overseas subscriptions should be made in US dollars, i.e. US\$76.00, or in your national currency based on the prevailing exchange rate to the US dollar of that currency, payable to the British Medical Association. Orders can also be placed locally through any leading subscription agent or bookseller. (For the convenience of readers in USA, subscription orders, with or without payment, can be sent to: *British Medical Journal,* Box 560B, Kennebunkport, Maine 04046. All inquiries, however, must be addressed to the publisher in London.) All inquiries regarding air mail rates and single copies already published should be addressed to the publisher in London.

NOTICE TO ADVERTISERS Applications for advertisement space and for rates should be addressed to the Advertisement Manager, *British Journal of Venereal Diseases,* BMA House, Tavistock Square, London WC1H 9JR.

COPYRIGHT © 1982 by the *British Journal of Venereal Diseases.* This publication is copyright under the Berne Convention and the International Copyright Convention. All rights reserved. Apart from any relaxations permitted under national copyright laws, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means without the prior permission of the copyright owners. Permission is not, however, required to copy abstracts of papers or of articles on condition that a full reference to the source is shown. Multiple copying of the contents of the publication without permission is always illegal.



ANNOUNCING...

The Fifth Meeting of the International Society for STD Research

Seattle, Washington U.S.A. August 1-3, 1983

SCIENTIFIC MEETING

August 1-3

This program will encompass the epidemiology, biology, pathogenesis, diagnosis and treatment of sexually transmitted diseases including:

1. *Neisseria gonorrhoeae*
2. *Chlamydia trachomatis*
3. Sexually transmitted viruses including herpes simplex virus, cytomegalovirus, human papilloma virus
4. *Treponema pallidum*
5. Infections in homosexual men including acquired immunodeficiency syndrome, enteric pathogens, hepatitis
6. STDs in women including vaginitis and urethritis syndromes, STDs in pregnancy and salpingitis/infertility
7. Relationship of STDs to malignancy
8. Other STD agents

SUPPLEMENTAL PROGRAMS

1. CLINICAL UPDATE — July 31, 1983
   This program will provide a clinical update of Genital HSV Infections, Lower Genital Tract Infections in Women, PID and its sequelae, Management of Urethritis, Proctitis-Enteritis syndromes and Acquired Immunodeficiency Syndrome in the homosexual patient.
2. CURRENT ASPECTS OF STDS SYMPOSIUM—III (CASTDS): Critical Gay Health Needs: New Diseases, Persistent Problems and Prophylaxis — August 4, 1983
   This program will focus on STDs and related health problems in homosexual men. It is independently sponsored by the National Coalition of Gay STD Services (N.C.G.S.T.D.S.).

PLEASE SEND: ☐ Program/Registration Form/Abstract Form for August 1-3 I.S.S.T.D.R. Scientific Meeting

☐ Information/Registration Form for July 31st Clinical Update

☐ Information/Registration Form/Abstract Form for the August 4th C.A.S.T.D.S. program

NAME (Please print or type)____________________

MAILING ADDRESS (include zip code)____________________

____

____

Send this portion to: PROGRAM COORDINATOR I.S.S.T.D.R., 325 - 9th Ave., Seattle, WA 98104

UNIVERSITY LIBRARY

M.S.S.V.D.

The Medical Society for the Study of Venereal Diseases

11 Chandos Street, Cavendish Square, London W1M 0EB
President: Dr. Jack Barrow

The object of the Society is to bring together those interested or engaged in the diagnosis, treatment, and public health aspects of venereal diseases. The Society meets regularly in London for the reading of papers, discussions, and presentation of cases. An out-of-London meeting is held in the Spring.

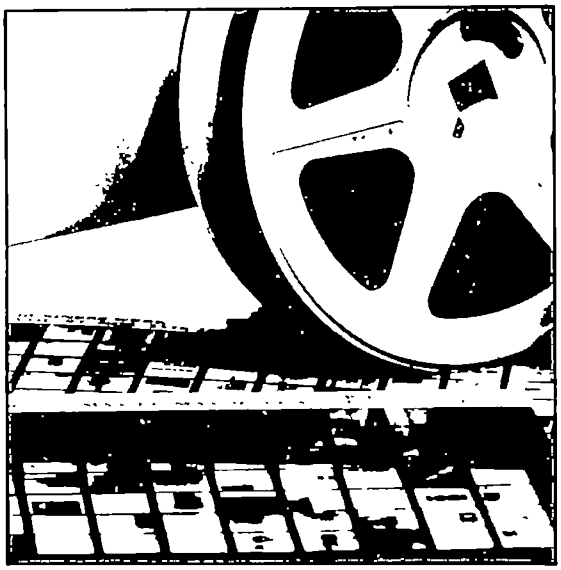
Annual subscription for membership of the Society is £20 for all members.
Applications for membership are very welcome and forms can be obtained from the Honorary Secretary, Dr. M. A. Waugh, Department of Genito-Urinary Medicine, The General Infirmary Great George Street, Leeds LS1 3EX.

Proposed Programme – 1983

Friday 28th January 1983
Prostatitis —'Per Rectal Ultrasound Scanning of the Prostate.
Speaker: Mr. W. B. Peeling, FRCS
Friday 25th March 1983 Proposed Meeting
The Gay Immuno Suppression Syndrome — clinical and epidemiological aspects
Speaker: Dr. Harold W. Jaffe, CDC, Atlanta

Friday 22nd April 1983
Presidential Address — Dr. Elisabeth Rees
Spring Meeting 1983 — 12th/16th May
Rotterdam, Netherlands

This Publication is available in Microform.



University
Microfilms
International

Please send additional information
for ______________________
(name of publication)
Name______________________
Institution______________________
Street______________________
City______________________
State______________ Zip__________

300 North Zeeb Road
Dept. P.R.
Ann Arbor, Mi. 48106

Br J Vener Dis 1982;**58**:351-4

Treponemal serology on Bali Island, Indonesia

R NEY,* M F GARNER,† J L BACKHOUSE,† N WIRIA DUARSA,‡
DARWATA BREGUET,‡ AND G BREGUET§

*From the *Department of Human Genetics, CHUV, Lausanne, Switzerland; the †Institute of Clinical Pathology and Medical Research, Sydney, Australia; the ‡Faculty of Medicine, University of Udayana, Denpasar, Bali, Indonesia; and the §Department of Anthropology, University of Geneva, Switzerland*

SUMMARY As part of a multidisciplinary study of the population of Bali, Indonesia, treponemal serology was carried out on 2452 serum samples from subjects of both sexes. Sera reactive to the *Treponema pallidum* immobilisation test (TPI) were found in 81 (3·3%) subjects with a male prevalence of 4% and a female prevalence of 2%. All the reactive sera were from villagers. Of 1118 students sampled in various towns, none had reactive TPI tests. The prevalence of reactive sera varied greatly from one village to another; up to 50% of the sera examined were reactive. Geographical and socioeconomic analyses of the data show a strict correlation between poor socioeconomic status and high reactivity rates to the TPI test. Fifty-seven per cent of all the reactive sera originated from subjects living in two districts where yaws had recently been reported. Only three of the 1406 subjects, aged 15-29 years, had reactive sera. The reactivity rate steadily increased in the age groups 30-44, 45-59, and 60 years and over. Biological false-positive reactions occurred in 3·8% of the sera tested.

Introduction

The island of Bali (115° longitude east/8°30 latitude south) is the first of the Lesser Sunda Islands from the west. Its surface area is 5600 km^2 and its climate tropical, with a dry and a rainy season but a relative humidity always above 70%. The average temperature is around 30°C, with variations between day and night not exceeding 6°C. There are important climatic variations between the north, rather dry part of the island, the wet mountainous centre, and the alluvial plains of the south. In 1980 the population was 2 500 000 inhabitants, with a density varying from 200 to 700 inhabitants/km^2. Most of the population practises a local Hindu religion and lives in traditional rural villages, the social structures of which have remained unchanged for many centuries.[1] The tolerance to premarital or extramarital intercourse varies greatly from one community to the other, but in most cases the sexual partners belong to the same community. Traditionally there are two kinds of marriages in Bali: the union arranged by the parents and the union of two persons in love after a ritual elopement; in the second case, a large proportion of the brides are pregnant.

Address for reprints: Dr R Ney, 1041 Bretigny-sur-Morrens, Switzerland

Accepted for publication 20 June 1982

The nutritional state of the population is generally adequate. Famine has not occurred in Bali in the last decade, except in the poor areas of the east and on the island of Nusa Penida (figure).

Yaws was present throughout Bali until eradication campaigns were carried out by the Indonesian Government in the late 1950s and early 1960s using penicillin.[2] These campaigns combined with the wide use of penicillin for other diseases, particularly in children, dramatically reduced the incidence of yaws. In 1980, 27 cases of yaws were reported to the Communicable Diseases Center of the Department of Health in Bali. All the cases were located in two specific areas (figure): the first one (Tejakula District) in the north-east of Bali, with one active and 10 non-active cases, the other on the island of Nusa Penida, with 15 active and one non-active cases. Both locations are among the poorest areas of Bali.

Syphilis is not endemic in Bali, probably because of its moist tropical climate. Venereal syphilis is reported in the two main towns, Denpasar and Singaraja, but its prevalence is difficult to evaluate, mainly owing to the lack of normal population samples. The only available data are those of the Communicable Diseases Center (1977-1979),[3] which runs regular clinical and serological surveys (using the Venereal Disease Research Laboratory test only) among particular groups: soldiers, students, government employees, workers emigrated from

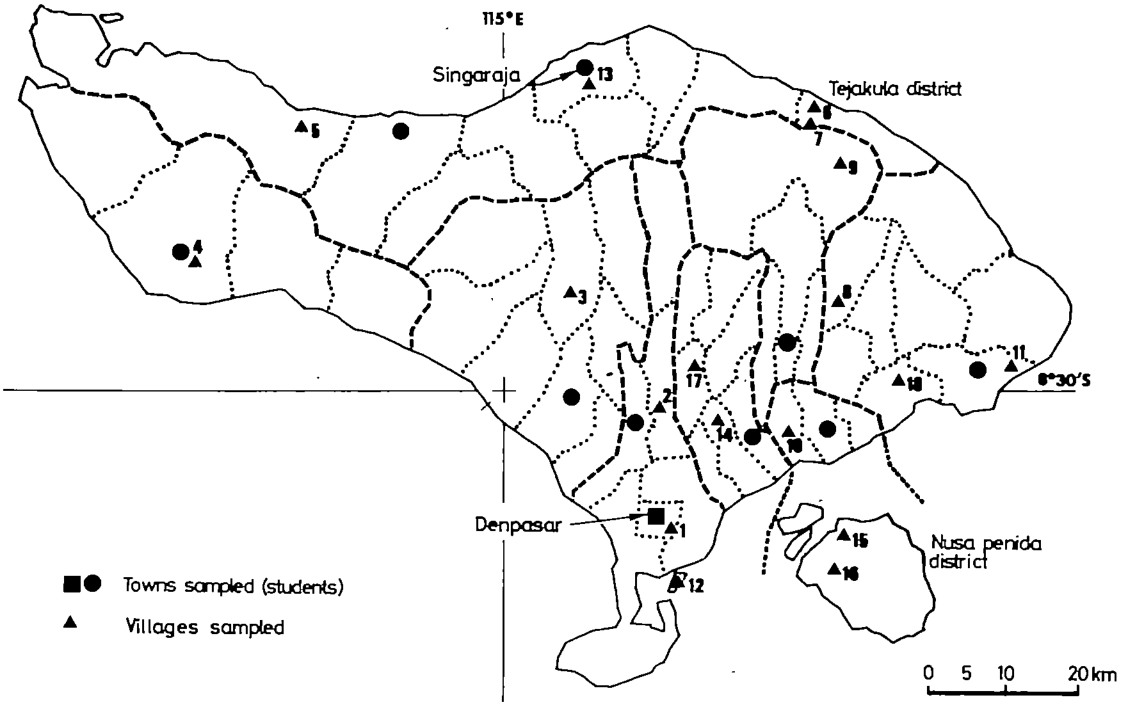


FIGURE *Map of Bali, Indonesia, showing the villages sampled: (1) Renon, (2) Ayunan, (3) Penebel, (4) Dauh Waru, (5) Grokgak, (6) Julah and Pacung, (7) Sembiran, (8) Rendang, (9) Songan, (10) Banjar Angkan, (11) Seraya, (12) Serengan, (13) Bratan, (14) Wanayu, (15) Ped, (16) Kelumpu, (17) Ubud, (18) Tenganan.*

villages and living in the town, and prostitutes. An average of 4000 sera were tested yearly, and the annual incidence of new cases was: 1977, 2·9%; 1978, 1·5%; and 1979, 0·9% of the population sampled.

Patients and methods

A multidisciplinary study of the Balinese population was undertaken between 1978 and 1981 by the department of anthropology of the University of Geneva, with the collaboration of the University of Udayana (Bali). The main goal was to study the blood polymorphism and the genetic differentiation of this population. One of the other projects undertaken was to determine the prevalence of treponemal infection in Bali.

POPULATION SAMPLE

Students (1118) of both sexes aged 16 to 29 (mean age 21·4 years for men, 20·6 years for women) were sampled in the 10 main towns of the island. Most were from upper middle-class or wealthy families and belonged to the high caste religious group. They rarely came from the poorest rural areas.

Village people (1334) of both sexes (mean age 40·8 years for the men and 40·7 for the women) were sampled in 19 different locations (figure) in all the regencies of the island. They were low-caste peasants living in rural areas. Their socioeconomic state was much lower than that of the students in the first group but showed great variation from village to village.

CLINICAL EXAMINATIONS

Subjects from whom blood was taken received medical care if they had any complaint, but a systematic clinical evaluation of treponematosis was not possible. The female and the male genitalia could rarely be examined. Many subjects had slight bowing of the tibiae, leg scars, or skin eruptions, but these clinical findings were difficult to interpret, especially in the older age group, as in many instances they could not be distinguished from conditions resulting from poor nutrition, previous injuries, or burns.

SEROLOGY

Blood was collected aseptically by venepuncture and sent as soon as possible by air to Switzerland, where the serum was separated and frozen within 48 hours of blood collection. Sera were sent frozen to the

Institute of Clinical Pathology and Medical Research, Westmead, Australia, for treponemal antibody testing. The following tests were carried out on each specimen: Venereal Disease Research Laboratory (VDRL) slide test, fluorescent treponemal antibody absorbed (FTA-ABS) test, and *Treponema pallidum* immobilisation (TPI) test. A reactive TPI test result was taken to indicate past or present treponemal infection and all other results were compared with it.

Results

TOTAL GROUP

Details of reactivity to the TPI test by age and sex are shown in table I. Of the 2452 sera examined, 81 (3·3%) were reactive. The mean age of the reactive subjects was 49·2 years; the mean age of the village population sampled was 40·8 years. The incidence of reactive subjects steadily increased with age and reached a maximum of 11·2% in the older age group.

TOWNS AND VILLAGES

None of the 1118 students tested were TPI reactive. This result should be compared with that of a group of 135 students from South Sulawesi (54 from Ujung Pandang and 81 from Makale), in which no sera were reactive to the TPI test. The 19 villages sampled have been grouped according to the increasing incidence of reactivity to the TPI test in table II. The incidence was higher among men than among women in all but two of the villages. In the age group 15-29 years the incidence for villagers only (without the students) was 1·0% (three reactive sera out of 288); 1·1% (two of 176) of the men and 0·9% (one out of 112) of the women had reactive sera. The only area where the results were appreciably different from the rest of Bali was the island of Nusa Penida, 15 km offshore from the mainland. It is a dry sandstone plateau inhabited by a population living a hard and poor life. The reactivity rates of this population in Kelumpu and Ped was 8·3% in the age group 15-29 years; 46·4% in the age group 30-44 years; 62·5% in the age group 45-59 years; and 83·3% in the age group 60 years and over. There was no difference in the incidence of reactivity between men and women (26 reactive sera from 52 men, and eight reactive sera from 18 women).

COMPARISON OF TEST RESULTS

For the purpose of this survey the TPI test was regarded as the most specific test for treponemal infection[4 5]; the results of other tests were compared with it. The number of sera reactive in each test were: TPI, 81; FTA-ABS, 92; and VDRL, 149. There were 49 sera which were reactive in all three tests. Only six of these showed VDRL titres of $\geqslant 8$. The mean age of these six subjects was 48·3 years, which was similar to the mean age of all those subjects with reactive sera in the TPI test (49·2 years).

All sera reactive in the TPI test were also reactive

TABLE I *TPI test results in relation to age and sex in population studied*

	Both sexes			*Men*			*Women*		
		TPI-reactive			*TPI-reactive*			*TPI-reactive*	
Age (years)	*No of sera tested*	*No*	*%*	*No of sera tested*	*No*	*%*	*No of sera tested*	*No*	*%*
0-14	22	0	0·0	14	0	0·0	8	0	0·0
15-29	1406	3	0·2	1021	2	0·2	385	1	0·3
30-44	491	26	5·3	278	20	7·2	213	6	2·8
45-59	345	31	9·0	202	23	11·4	143	8	5·6
60-+	188	21	11·2	127	20	15·7	61	1	1·6
Total	2452	81	3·3	1642	65	4·0	810	16	2·0

TABLE II *Number and percentage of sera reactive to the TPI test by sex in the village populations*

		Both sexes			*Men*			*Women*		
			TPI-reactive			*TPI-reactive*			*TPI-reactive*	
% of TPI reactivity	*Villages**	*No of sera tested*	*No*	*%*	*No of sera tested*	*No*	*%*	*No of sera tested*	*No*	*%*
0	5, 8, 13, 14, 17	320	0	0·0	179	0	0·0	141	0	0·0
1-5	1, 3, 7, 9, 10, 18	609	17	2·8	356	16	4·5	253	1	0·4
6-20	2, 4, 6, 11, 12	335	30	9·0	210	23	11·0	125	7	5·6
+20	15, 16	70	34	48·6	52	26	50·0	18	8	44·4

*See figure for location of villages corresponding to the numbers given in the FTA-ABS test. Of these 81 sera, 49 were reactive and 32 non-reactive in the VDRL test. The results of the TPI and FTA-ABS tests were in disagreement in 11 sera, in which the FTA-ABS test was reactive and the TPI test non-reactive. The VDRL results in these 11 sera were reactive in seven and non-reactive in four. The mean age of these 11 subjects was 43·0 years. Biological false-positive reactions, with only the VDRL being reactive, were found in 92 (3·8%) of the 2452 sera tested; the mean age of these subjects was 36·8 years.

Discussion

Despite the inadequacy of serological tests to differentiate between infection with yaws and syphilis, the results of this survey provide some useful information. No sera were reactive to the TPI test in the small group of children and in almost all of the large group of young adults (15-29 years). Yaws is mainly a disease of children, and thus these groups had presumably not been exposed to this infection; this indicates that the yaws campaigns nearly eradicated the disease 25 years ago. Another important fact is that these groups are also free of venereal syphilis. There is a steady increase in the reactivity rate to the TPI test in the age groups 30-44, 45-59, and over 60 years. This could indicate a spontaneous decrease in the incidence of yaws in the 1940s and 1950s before the penicillin treatment campaigns, as was observed for syphilis in the United Kingdom.[6]

The geographical distribution of the villages with a high reactivity rate to the TPI test corresponded with the two areas where active yaws had recently been reported (personal communication). There was also a distinctive correlation between poverty and a high reactivity rate. The population of these villages was the most unlikely to have had contact with the possible foci of syphilis in the two main towns, Denpasar and Singaraja.

Unlike other Asian towns prostitution associated with tourism is almost non-existent in Bali.

The low proportion of TPI-reactive subjects with high VDRL titres suggests that latent or previous inactive treponemal infection was prevalent among the TPI-reactive subjects. The analysis of the data strongly suggests that most of them had been infected with yaws in the past. The incidence of venereal syphilis was probably very low in the population tested.

The problem of sera reactive in the FTA-ABS but non-reactive in the TPI test is difficult. Mackey *et al*[5] concluded that the FTA-ABS test was more sensitive than the TPI test, particularly in treponemal infections of long duration, but that its specificity was not absolute.

Biological false-positive reactions occurred frequently in this survey (3·8%) whereas Jegathesan *et al*[7] found an incidence of 1·3% among 10 096 expectant mothers in Malaysia and the incidence among 21 924 sera at the Institute of Clinical Pathology and Medical Research, Westmead, Australia, was 0·2%.[8] In the survey on Kar Kar Island, New Guinea, Garner *et al*[9] found a rate of 1·7%. The causes of biological false-positive reactions are multiple.[7] Possibly the difference in rates in the age group 16-29 years between men (2·0%) and women (11·2%) is due to repeated pregnancies in women.

This survey was funded by the Swiss National Fund for Scientific Research (grant 3888) and partially assisted by a grant from the World Health Organisation (grant G3/181/105A). We are grateful to the Indonesian Institute of Sciences (LIPI) for its help and support.

References

1. Ramseyer U. *The Art and Culture of Bali.* London: Oxford University Press, 1977.
2. Harahap M. Sexually transmitted diseases in Indonesia. *Br J Vener Dis* 1980;**56**:282-4.
3. Directorate of Communicable Diseases Center. *Annual Report of the Department of Health of the Province of Bali (1978-1979),* Bali, 1980.
4. Wilkinson AE, Rayner CFA. Studies on the fluorescent treponemal antibody (FTA) test. *Br J Vener Dis* 1966;**42**:8-15.
5. Mackey DM, Price V, Knox JM, Scotti A. Specificity of the FTA-ABS test for syphilis. *JAMA* 1969;**207**:1683-5.
6. Blount JH, Holmes KK. Epidemiology of syphilis and non-venereal treponematosis. In: Johnson RC, ed. *The Biology of Parasitic Spirochetes.* New York: Academic Press, 1976: 157-76.
7. Jegathesan M, Fan YH, Ong KJ. Seroreactivity to syphilis in Malaysian blood donors and expectant mothers. *South-east Asian J Trop Med Pub Hlth* 1975;**3**:413-8.
8. Garner MF. The biological false-positive reactions to serological tests for syphilis in blood donors. *J Clin Pathol* 1970;**23**:31-4.
9. Garner MF, Hornabrook RW, Backhouse JL. Prevalence of yaws on Kar Kar Island, New Guinea. *Br J Vener Dis* 1972;**48**:350-5.

Br J Vener Dis 1982;**58**:355-8

Syphilis in pregnant women in Zambia

ATTILI V RATNAM, SHAHIDA N DIN, SUBHASH K HIRA, GANAPATI J BHAT, D S O WACHA, A RUKMINI, AND RAPHAEL C MULENGA
From the University Teaching Hospital, Lusaka, Zambia

SUMMARY Because of the high incidence of congenital syphilis at the University Teaching Hospital, Lusaka, Zambia, the potential risks of congenital infection and fetal loss due to syphilis were assessed by screening 202 antenatal patients, 340 pregnant women admitted to the hospital whose pregnancies ended in either spontaneous abortion or stillbirth, and 469 consecutive babies delivered at the hospital. Primary serological screening was performed with the rapid plasma reagin test, and reactive sera were confirmed by the *Treponema pallidum* haemagglutination test. In all cases detailed histories were obtained and patients were examined for clinical signs of syphilis. The TPHA test result was reactive in 12·5% of antenatal patients and in 42% of women who aborted in the later half of pregnancy. Among 469 consecutive babies delivered at the hospital, 30 had reactive results to the TPHA test; of these two were stillborn and four had signs of congenital syphilis at birth. Thus, syphilis appears to affect adversely an appreciably high number of pregnant women in Zambia. For this reason a special campaign to screen adequately and treat pregnant women and neonates is needed.

Introduction

There have been no reports of the existence of non-venereal treponematoses in Zambia, and no cases have been suspected among more than 60 000 patients seen in a five-year period at the dermato-venereology department of the University Teaching Hospital, Lusaka. The reason for this is the near temperate climate of Zambia with temperatures not exceeding 32°C and a light rainfall, which is limited to only four months in the year. On the other hand, venereal syphilis is a very common problem seen at the University Teaching Hospital in Lusaka, the capital city, and also at sexually transmitted disease (STD) clinics in other regions of the country. Despite a ratio of 5 : 1 of male and female patients attending the STD clinic at this hospital the number of women with syphilis is twice that of men; they are mostly seen in the later secondary stage of infection.[1] Early syphilis was diagnosed in 7·6% of pregnant women attending this clinic, and as a result of routine screening in the antenatal clinic of this hospital 17·9% of women had reactive serological test results for syphilis.[2] Consistent with the high prevalence of syphilis in pregnant women, congenital syphilis was common (8·6%) among infants under 3 months of age who required admission to hospital.[3]

These preliminary investigations suggested a pool of infectious syphilis in women of reproductive age with high risks of neonatal infection and pregnancy loss. The present study was undertaken to assess the extent of this problem and to identify risk factors that might be eliminated by improved prenatal care.

Patients and methods

Three groups of patients were investigated: (a) 202 pregnant women who attended a suburban antenatal clinic for the first time; (b) 340 pregnant women whose pregnancies resulted in either spontaneous abortion or stillbirth; and (c) 464 pregnant women admitted to the labour ward of this hospital for delivery. In each group consecutive patients were examined. The socioeconomic background and relevant obstetric and prenatal histories of each patient were recorded; the patients were examined for clinical signs of syphilis. Examination by darkfield microscopy was performed in appropriate cases. Blood samples were obtained for preliminary serological screening for treponemal antibodies with the rapid plasma reagin (RPR) test and reactive sera were tested by the *T pallidum* haemagglutination (TPHA) test according to the manufacturers' instructions (Beckton, Dickinson & Co and

Address for reprints: Dr A V Ratnam, Dermatovenereology Division, University Teaching Hospital, PO Box RW 50001, Lusaka, Zambia

Accepted for publication 10 May 1982

Wellcome Research Laboratories respectively). In the third group of patients preliminary screening was performed on cord blood, and if that was reactive blood samples from both mother and baby were obtained for testing by the RPR and TPHA tests. In addition, in all babies whose sera were reactive in these tests clinical and radiological examination of the long bones was performed. The cerebrospinal fluid was examined routinely for the presence of treponemal antibodies.

Results

The results of serological testing of patients in the three groups are shown in table I. At the time of examination 85% of the women in the antenatal group were ≥20 weeks pregnant. One hundred and seventy-three of the 240 spontaneous abortions occurred before the 20th week of pregnancy. Seroreactivity in the TPHA test in these two subgroups was 9·8% and 41·8% respectively. In 36 of the 42 seroreactive women who gave birth to stillborn babies, the presence of syphilis was the only recognisable aetiological factor. Thirty-five women had premature deliveries; 40 of the babies were macerated at birth.

The marital state and antenatal and obstetric histories of the 167 cases of fetal loss beyond the 20th week of pregnancy are given in table II. There were no significant differences in age, marital and economic state, education, and parity between seropositive and seronegative groups. A history of previous abortion or stillbirth and antenatal reactivity to the Venereal Disease Research Laboratory test was significant in the seroreactive group. A similar analysis of 464 consecutive pregnant women at delivery is shown in table III. Among the 30 seroreactive babies, four had clinical signs of congenital syphilis at birth (*T pallidum* was detected by darkfield microscopy of exudate in three babies), two were stillborn and, because of prematurity, asphyxia, and conjunctivitis, a further eight required intensive care. The overall mortality and morbidity rates in babies whose sera were reactive in the TPHA test were significantly higher ($\chi^2 = 68\cdot86$; $P < 0\cdot001$) than in those whose sera were not reactive.

Discussion

The results of this study confirm that syphilis is a major problem in Zambia with a high risk of pregnancy loss and congenital infection. Four of 459 consecutive liveborn babies had signs of congenital syphilis at birth. The actual incidence can be expected to be much higher among the 30 seroreactive neonates even though some might have acquired maternal antibodies only by passive transfer. In view of the potential risk all the 30

TABLE I *Results of testing sera by the rapid plasma reagin (RPR) and T pallidum haemagglutination (TPHA) tests for treponemal antibodies in three groups of patients*

Group of patients	*No of sera reactive by RPR test*	*No of sera confirmed reactive by TPHA test (% of total screened)*	*Total No of sera screened*
Antenatal patients	29	25 (12·5)	202
Patients whose pregnancies resulted in:			
Spontaneous abortions	52	45 (18·8)	240
Stillbirths	45	42 (42·0)	100
Patients admitted for delivery	33	30 (6·5)	464

TABLE II *Marital state, obstetric history, and antenatal screening results by RPR test in 167 women who had a spontaneous abortion after the 20th week of pregnancy or a stillbirth*

	No of women with the following TPHA test results:		
Marital state and obstetric history	*Seronegative (n = 97)*	*Seropositive (n = 70)*	*Total (%) (n = 167)*
Unmarried	8	11	19 (11·4)
Past history of abortion/stillbirth	7	12	19 (11·4)
Non-attendance at antenatal clinics	34	26	60 (35·9)
Antenatal VDRL results at initial attendance			
Test not performed	65	60	125 (74·9)
Reactive	0	5*	5 (11·9)
Non-reactive	32	5	37 (88·1)

*No specific treatment received
VDRL = Venereal Disease Research Laboratory (test); TPHA = *T pallidum* haemagglutination (test)
RPR = rapid plasma reagin (test)

TABLE III *Marital state, obstetric history, results of antenatal screening by the RPR test, and outcome of pregnancy in 464 women admitted for delivery*

	No of women with the following TPHA test results:		
Marital state and obstetric history	*Seronegative (n = 434)*	*Seropositive (n = 30)*	*Total (%) (n = 464)*
Unmarried	18	1	19 (4·1)
History of abortion/stillbirth	59	8	67 (14·4)
Non-attendance at antenatal clinics	60	8	68 (14·7)
Antenatal VDRL results at initial attendance			
Test not performed	110	2	112 (28·3)
Reactive	5	10	15* (5·3)
Non-reactive	259	10	269 (94·7)
Outcome of delivery†			
Stillbirth	3	2	5 (1·1)
Baby required intensive care	19	12	31 (6·1)
Healthy baby	418	16	434 (93·5)

*Only 11 women had received treatment before delivery
†Total number of babies was 470, which included six pairs of twins

seroreactive babies were given treatment. In a similar study in Addis Ababa[4] seroreactive babies at birth were followed up without treatment and 21·3% subsequently developed signs and symptoms of early congenital syphilis.

Women with syphilis abort more than 60% of their pregnancies,[5] which might explain the much lower seroreactivity rate among neonates compared with that of the prenatal women. The fact that there was a high incidence of clinical syphilis and seroreactivity in cases of fetal loss in the later half of pregnancy supports this view. In the Johns Hopkins Hospital in 1923 syphilis was found to be the aetiological agent in 40% of stillborn premature infants and in about 80% of macerated babies.[6] It is surprising that our results compare with those in the preantibiotic era. A high seroreactivity rate in women aborting in the later half of pregnancy as well as in women producing stillbirths compared with a lower rate of 9·8% in women aborting in early pregnancy supports the widely accepted view that syphilis is a major factor in fetal loss only in the later half of pregnancy.[7] It is now increasingly believed, however, that fetal infection does occur even in the early weeks of pregnancy but appreciable damage to the fetus results only later in the pregnancy when an adequate immune response has developed in the fetus.[8-10]

Considering the high prevalence of syphilis in Zambia, the present antenatal care appears to be far from adequate (table III). Despite the fact that 85% of women had attended one or more antenatal examinations, screening by the VDRL test was not carried out in more than one-quarter of these women; furthermore, not all those with positive results received treatment. The problem of women acquiring syphilis after screening by the VDRL test in early pregnancy has been highlighted before,[11] and the inadequacy of one serological test in early pregnancy in a high risk population is apparent in our study. Ten women who had non-reactive test results in early pregnancy were seroreactive at the time of delivery. Similarly, in cases of spontaneous abortions in the later half of pregnancy, five had initially negative results. The presence of active early syphilis in as many as 26 out of 70 women in the later half of pregnancy also indicated recently acquired infection. A significantly greater number of these women had had a previous spontaneous abortion and they may carry a higher risk of repeated abortions. Since most pregnant women in Zambia attend for their first antenatal examination when the pregnancy is fairly advanced (⩾20 weeks) the screening and treatment of women who might acquire syphilis early in pregnancy, which subsequently ends in an abortion, can be a difficult task. Under the present circumstances epidemiological treatment of all pregnant women with a history of a previous abortion in the later half of pregnancy would be justified. The absence of significant differences in age, education, parity, and socioeconomic factors between the seropositive and seronegative groups probably indicates widespread public ignorance of the problem.

The dramatic decline in the incidence of venereal syphilis after the introduction of penicillin in the mid-1940s and its slow resurgence in the past two decades is well known in the developed countries where statistics are available. Such information is not, however, available in the African continent and, especially in countries where non-venereal treponematoses existed at one time or other, the general situation remains unclear. Nevertheless, very high rates of venereal syphilis have been reported in urban areas in many African countries.[12-18] Seroreactivity rates among pregnant women and the incidence of congenital syphilis are also staggeringly high[3 4 19 20] compared with those in the developed world.[21-23] Despite the slow resurgence in the developed countries the incidence of syphilis (except in homosexuals) has been low, and increasing concern is now felt about the complacency of the medical profession and the attendant risk of diagnostic failure.[24 25] Unfortunately, similar attitudes prevail in African countries which disregard the sustained high prevalence of syphilis in local populations. All grades of the medical profession in Africa, especially those concerned in maternal and infant care, need to be reminded that in our environment syphilis continues to behave as in the preantibiotic era, and therefore screening and early treatment of pregnant women is imperative.

Of the many congenital infections syphilis is not only the most readily identifiable but also the most easily treated. Hence there is an urgent need to revitalise antenatal care in Zambia with sufficient focus on syphilis. For this purpose we recommend that:

(1) Every pregnant woman has a screening test for syphilis as soon as pregnancy is diagnosed and again in late pregnancy to detect infection acquired subsequently;
(2) Because babies born of women who do not have antenatal care are at high risk for congenital syphilis, cord blood should be screened; and
(3) Because a small number of women may not seek health care either for antenatal examination or for delivery, their infants should be screened for syphilis at the earliest opportunity.

To achieve these objectives a greater awareness among health staff as well as the general public is essential and a nationwide special campaign is recommended.

References

1. Ratnam AV. Sexually transmitted diseases in Lusaka. *Med J Zambia* 1980; **14:**71-4.
2. Ratnam AV, Chatterjee TK, Mulenga RC. Sexually transmitted diseases in pregnant women. *Med J Zambia* 1980; **14:** 75-8.
3. Hira SK, Ratnam AV, Sehgal DB, Bhat GJ, Mulenga RC. Congenital syphilis in Lusaka. I Incidence in a nursery ward. *E Afr Med J* 1982; **59:**241-6.
4. Larsson Y, Larsson U. Congenital syphilis in Addis Ababa. *Ethiopian Med J* 1970; **8:**163-72.
5. Royston GD. A statistical study of the causes of abortion. *Am J Obstet* 1917; **76:**571-84.
6. Williams JW. *A textbook of obstetrics*, 6th ed. New York: D Appleton & Co, 1923.
7. Dippel AL. The relationship of congenital syphilis to abortion and miscarriages, and the mechanism of intrauterine protection. *Am J Obstet Gynecol* 1944; **47:**369.
8. Harter CA, Benirschke K. Foetal syphilis in the first trimester. *J Obstet Gynaecol* 1976; **124:**705-11.
9. Hager WD. Transplacental transmission of spirochetes in congenital syphilis. *Sex Transm Dis* 1978; **5:**122-3.
10. Benirschke K. Syphilis—the placenta and the fetus. *Am J Dis Child* 1974; **128:**142-3.
11. Monif GRG, Williams BR, Shulman ST, Baer H. The problem of maternal syphilis after serological surveillance during pregnancy. *Am J Obstet Gynecol* 1973; **117:**268-70.
12. Lomholt G. Venereal problems in a developing country. *Trop Doct* 1976; **6:**7-10.
13. Arya OP, Ongom VL, Tomusange ET. The role of a rural health centre in the control of venereal diseases in Uganda. *E Afr Med J* 1974; **51:**109-21.
14. Arya OP, Taber SR. Some observations on venereal disease and infertility in the Teso district of Uganda. In: Adadevoh BK, ed. *Report of an International Workshop on Correlates of Subfertility and Infertility in Africa.* Ibadan, Nigeria: Caxton Press Ltd, 1974:78-80.
15. Friedmann PS, Wright DJM. Observations on syphilis in Addis Ababa. II Prevalence and natural history. *Br J Vener Dis* 1977; **53:**276-80.
16. Dogliotti M. The incidence of syphilis in the Bantu: survey of 587 cases from Baragwanath Hospital. *South Afr Med J* 1971; **45:**8-10.
17. Willcox RR. Venereal syphilis and other venereal infections in non-venereal treponematoses areas in Africa. *Central Afr Med J* 1958; **4:**432-43.
18. Ursi JP, Dyck van E, Houtte van C, Piot P, Colaert J, Meheus A. Syphilis in Swaziland: A serological survey. *Br J Vener Dis* 1981; **57:**95-9.
19. Bwibo NO. Congenital syphilis. *E Afr Med J* 1971; **48:**185-8.
20. Cremin BJ, Fisher RH. The lesions of congenital syphilis. *Br J Radiol* 1970; **43:**333-9.
21. Grossman J III. Congenital syphilis. *Teratology* 1977; **16:** 217-24.
22. Hare MJ. Serological tests for treponemal disease in pregnancy. *J Obstet Gynaecol Br Commonwealth* 1973; **80:** 515-9.
23. Salo OP, Aho K, Nieminer E, Hormila P. False-positive serological tests for syphilis in pregnancy. *Acta Derm Venereol* 1969; **49:**332-7.
24. Bueno M, Perez-Gonzalez J, Saria A. Congenital syphilis. *Paediatrician* 1979; **8:**17-34.
25. Gingrich RD, Drusin LM, Kavey R-EW. Early congenital syphilis. *NY State J Med* 1976; **76:**283-8.

Br J Vener Dis 1982; 58: 359-62

Detection of Neisseria gonorrhoeae antigen by a solid-phase enzyme immunoassay

H A AARDOOM,* D DE HOOP,† C O A ISERIEF,† M F MICHEL,* AND E STOLZ†
*From the *Department of Clinical Microbiology and Antimicrobial Therapy, and the †Department of Dermatovenereology, Erasmus University Rotterdam, Rotterdam, The Netherlands*

SUMMARY The Gonozyme test (Abbott Laboratories) is a new enzyme immunoassay for detecting *Neisseria gonorrhoeae* antigens in specimens from the urethra in men and the endocervix in women. To evaluate the usefulness of the assay 249 patients were investigated. The results obtained with the immunoassay were compared with those of culture and microscopy of Gram-stained smears. The sensitivity and specificity of the test were high in men with urethritis and acceptable in different groups of women. As the sensitivity of the Gonozyme test was much higher than that of microscopy for endocervical specimens it might be useful as a rapid screening test for the detection of gonorrhoea in women.

Introduction

The diagnosis of gonorrhoea is usually based on the detection of Gram-negative diplococci in Gram-stained smears and the isolation of *Neisseria gonorrhoeae* from urethral or endocervical material. A gonococcal infection might, however, also be detected by the presence of antigen in material from infected patients. For this purpose the Gonozyme test (Abbott Laboratories) has been developed; it can be read in three hours and uses a solid-phase enzyme immunoassay for the detection of gonococcal antigen in material from the male urethra and the endocervical canal of women. Because of possible cross-reactions with *Neisseria lactamica* and *Neisseria meningitidis* the testing of rectal and pharyngeal material by immunoassay is not recommended.

In the present study appropriate specimens for Gram-staining, culture, and the Gonozyme test were collected from four well-defined groups of patients attending a sexually transmitted diseases (STD) clinic.

Patients and methods

REAGENTS

The Gonozyme diagnostic kit consists of the following reagents: beads pretreated to facilitate absorption of *N gonorrhoeae* antigen; antibody to *N gonorrhoeae* obtained by injecting different gonococcal strains into rabbits[1]; antibody-enzyme conjugate, the antibody having been prepared by immunising goats with rabbit IgG and the enzyme used being horseradish peroxidase; a positive and negative control; and specimen storage solution and specimen dilution buffer. O-phenylenediamine 2HCl (OPD) was used as the substrate in the enzyme immunoassay.

STUDY GROUPS

Two hundred and forty-nine patients were investigated and divided into four groups: (A) 52 men with symptoms of urethritis; (B) 54 female contacts of patients suspected of having a gonococcal infection; (C) 41 patients (15 men and 26 women) who were re-examined after treatment for gonorrhoea; and (D) 102 prostitutes who regularly attended the STD clinic for the control of STDs.

COLLECTION OF SPECIMENS

Using a bacteriological loop material for Gram-staining was obtained from the male urethra and the endocervical canal of women. A second swab was used to collect specimens for gonococcal culture and for the immunoassay. As calcium alginate and the glue of wood may elicit false-positive reactions in the assay cottonwool-tipped plastic swabs were used in this study.

A chocolate agar plate and a Thayer-Martin plate were inoculated immediately after the collection of the specimen and, using a loop, the inoculum was spread over the plates. After incubation the density of the growth was graded from 1 to 4. After

Address for reprints: Professor Dr M F Michel, Department of Clinical Microbiology and Antimicrobial Therapy, Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands

Accepted for publication 30 May 1982

inoculation of the plates the swab was immersed in 0·2 ml of specimen storage solution in a 15-ml conical centrifuge tube; the tube was stored at 4°C. Before the assay was performed 1 ml of specimen dilution buffer was added to the tube. After 2-5 minutes the swab was rotated vigorously for 15 seconds, expressed against the wall of the tube, and discarded. All assays were performed within 24-48 hours of specimen collection.

PERFORMANCE OF ASSAY

Wells of a tray were filled with 0·2 ml of the specimen or appropriate control. A bead was then dispensed into each well and after incubation of the covered tray for 15 minutes at 37°C the beads were washed three times with distilled water. The next two steps consisted of the addition of 0·2 ml of antibody to *N gonorrhoeae* and 0·2 ml of antibody-enzyme conjugate. Each of these steps was followed by incubation of the trays and washing of the beads as described. After the last washing all excess water was removed from the wells and each bead was transferred to a tube; 0·3 ml of freshly prepared OPD solution was added to each tube and the tubes were left at room temperature for 10 minutes. The enzyme reaction was stopped by the addition of 1 ml of NHCl to the tubes containing a bead and to the substrate blank. After mixing the tubes were read in a spectrophotometer at 492 nm. Specimens giving values greater than 1·1 times the cutoff value were considered positive for *N gonorrhoeae*. The cutoff value was defined as a reading of 0·15 plus the mean of at least two negative controls. Values within 10% of the cutoff value were considered to be doubtful results.

COMPARISON OF THE ASSAY SCORES

As false-negative results can occur the findings on microscopy or culture cannot be used as absolute criteria for the existence of a gonococcal infection.[2 3] For this reason the results obtained with cultures and immunoassays were compared. From these comparisons two sensitivities and two specificities could be deduced. The same indices could be estimated when the results of microscopy and immunoassays were compared. According to the formula in table I the sensitivities and specificities were defined as follows: when the results of microscopy and culture were compared with those of immunoassay sensitivity equalled a/x and specificity d/y; when results of immunoassay were compared with those of culture or microscopy sensitivity was defined as a/p and specificity as d/q. In groups with negative microscopy or culture results and positive assay results and in those with positive microscopy or culture results but negative assay results the results were discordant. The figures of the discordant results were used for the estimation of the degree of misclassification, this index being defined as (b + c)/z.

TABLE I *Formula for comparing sensitivity and specificity of Gonozyme immunoassay with those of microscopy and culture*

	Immunoassay results	
Culture/microscopy results	*Positive*	*Negative*
Positive	a	b
Negative	c	d

a + b = x; c + d = y: a + c = p; b + d = q; total number x + y = p + q = z

Results

COMPARISON OF IMMUNOASSAY AND CULTURE

The results of the immunoassays and cultures in each of the four groups of patients and in all patients are given in table II. The mutual sensitivities and

TABLE II *Comparison of the sensitivities and specificities of the immunoassay and bacterial culture in four groups of patients*

	Patient groups									
	A		*B*		*C*		*D*		*All patients*	
	No	%*	*No*	%*	*No*	%*	*No*	%*	*No*	%*
Assay *vs* culture										
Sensitivity	35/35	90-100	13/15	60-98	2/3		11/12	62-100	61/65	85-98
Specificity	17/17	81-100	35/39	76-97	36/38	82-99	87/90	91-99	175/184	90-98
Culture *vs* assay										
Sensitivity	35/35	90-100	13/17	50-93	2/4		11/14	49-95	61/70	77-94
Specificity	17/17	81-100	35/37	82-99	36/37	86-100	87/88	94-100	175/179	94-99
Misclassification	0/52	0-7	6/54	4-23	3/41	2-20	4/102	1-10	13/249	3-9

*95% confidence intervals are given as probability percentages specificities and the degree of misclassification with both methods are shown.

Group A—In none of the 52 patients were the results discordant; the sensitivities and specificities were high.

Group B—Of the 54 patients, the immunoassay results were negative in two patients in whom *N gonorrhoeae* was isolated on culture and positive in four patients from whom the organism was not cultured. The 95% confidence limits of the sensitivities and specificities were dependent on the number of patients in the group (table II).

Group C—Of the 41 patients, the assay result was negative in one patient from whom *N gonorrhoeae* was cultured and positive in two patients in whom the organism was not isolated; the specificities were high.

Group D—Of the 102 women, the immunoassay was negative in one infected patient and positive in three patients in whom the culture results were negative.

Of the total 249 patients the assay results were negative in four patients from whom *N gonorrhoeae* was isolated and positive in nine patients in whom the organism was not cultured. The 95% confidence limits of sensitivities and specificities for all patients were high.

COMPARISON OF IMMUNOASSAY AND MICROSCOPY

The results of the immunoassays and microscopy are given in table III. In six patients (two from group A, two from group B, and two from group D) Gram-stained smears were not prepared and were not included in the following analysis.

Group A—Of 50 patients, the assay was positive in one in whom the microscopy result was negative.

Group B—Of 54 patients, typical organisms were seen on the smear of exudate from one patient in whom the assay result was negative. The results of the assay for 16 patients in whom Gram-negative diplococci were not seen were positive. When compared with the immunoassay the sensitivity of microscopy was low.

Group C—In three of 39 patients in whom Gram-negative diplococci were not seen the assay was positive. Specificities were high.

Group D—The assay results were positive in 13 of 100 women in whom Gram-negative diplococci were not seen on microscopy. Compared with the immunoassay the sensitivity of microscopy was low and the specificity was high.

Only one of the 243 patients had negative assay results but positive results on microscopy. In 33 patients the assay was positive although microscopy of a Gram-stained smear was negative. When compared with microscopy the sensitivity and specificity of the assay was high.

DISCORDANT RESULTS WITH CULTURE AND IMMUNOASSAY

Although the immunoassay results were negative *N gonorrhoeae* was isolated from material from four patients. In eight women and in one man gonococci were not cultured but the immunoassay results were positive. Because some uncommon gonococcal strains might have remained undetected by the Gonozyme test the following experiment was devised. Serial 10-fold dilutions of dense suspensions of each of four isolates were made in trypticase soy broth. After a sample had been taken for counting a swab was immersed in each tube and used for semi-quantitative cultures and immunoassay. The results are shown in table IV. The growth densities and the assay readings obtained with the clinical specimens are compared with those of the semiquantitative culture. The results from the clinical specimens corresponded fairly well with those obtained in the experiment; the sensitivity of the immunoassay (case Nos 1 and 16) was higher for some isolates than others (case Nos 2 and 15).

GROWTH-DENSITY OF CULTURES

The mean growth density of gonococcal cultures in group A was 3·5, in group B 2·1, in group C 2·4, and in group D 2·5.

TABLE III *Comparison of the sensitivities and specificities of the immunoassay and microscopy in four groups of patients*

	Patient groups									
	A		*B*		*C*		*D*		*All patients*	
	No	%*	*No*	%*	*No*	%*	*No*	%*	*No*	%*
Assay *vs* microscopy										
Sensitivity	33/33	89-100	1/2		1/1		1/1		36/37	86-100
Specificity	16/17	71-100	35/52	55-81	35/38	79-98	86/99	79-93	173/206	78-88
Microscopy *vs* assay										
Sensitivity	33/34	85-100	1/17	0·1-29	1/4		1/14	0·2-34	36/69	40-64
Specificity	16/16	79-100	36/37	86-100	35/35	90-100	86/86	96-100	173/174	96-100
Misclassification	1/50	0·1-11	17/54	19-45	3/39	2-21	13/100	7-21	34/243	10-20

*95% confidence limits are given as probability percentages

TABLE IV *Comparison of the results of bacterial culture and immunoassay of four gonococcal isolates from infected patients or from serial dilutions in broth*

Clinical isolates			*Serial dilutions*		
Case No	*Growth density*	*Immunoassay*† *reading*	*Growth density*	*Immunoassay*† *reading*	*No of organisms/ml in broth*
2	3	0·056	4	0·183	$1·0 \times 10^5$‡
15	1-2	0·587	2	0·196	$2·7 \times 10^4$‡
1	1	0·105	1	0·300	$6·0 \times 10^3$§
16	1-2	0·945	<1	0·475	$1·5 \times 10^2$§

†Reading >0·17 is positive and <0·13 is negative
‡Minimum number of gonococci per ml TSB giving a positive result in the immunoassay
§Inoculum giving a visible density on agar of ≮1 and a positive reaction in the immunoassay

Discussion

In relation to bacterial culture the sensitivity and specificity of the Gonozyme test for the detection of gonorrhoea in men with signs and symptoms of urethritis were high. Both indices were lower, however, in the other groups of patients studied. Several factors may explain these differences. It is reasonable to expect that the sensitivity of the immunoassay is related to the number of organisms in the collected specimen. On a scale of 1 to 4 the mean growth density of the cultures in group A was between grades 3 and 4. In women with gonococcal cervicitis the growth density was lower and varied between grades 2 and 3. The finding in four patients in whom gonococci were cultured but in whom the immunoassay results were negative or doubtful indicates the inherent risk if bacterial culture is replaced by the Gonozyme test. This risk should, however, be weighed against the nine (mainly female) patients who had positive results in the immunoassay but negative culture results. An explanation for this discrepancy may be that many patients in groups B and D regularly used a povidone-iodine douche. Although discordant results might be due to treatment with an antimicrobial agent before consultation none of the patients with discordant results between the Gonozyme test and bacterial culture had received these drugs before attendance at the clinic.

Microscopy of Gram-stained smears and the Gonozyme test can be considered as rapid tests for the detection of gonorrhoea. From a comparison of the results of both techniques the Gonozyme test was much more sensitive as a diagnostic test than microscopy in all groups except group A, in which these indices were high and equal. This is illustrated by the very low confidence limits of sensitivity of microscopy compared with those of immunoassay in patients in groups B and D (0·1-29% and 0·2-34% respectively).

The importance of the availability of a rapid test that is reasonably sensitive and specific hardly needs to be stressed. Takeguchi[4] developed a test based on the detection of an enzyme produced in appreciable amounts only by *N gonorrhoeae* and *Acinetobacter* spp; in women the test had a sensitivity of 62-88% and a specificity of 48-71%. These figures are lower than those of the Gonozyme test in women.

We thank Professor R van Strik of the Department of Biostatistics for his advice and Mrs A L Beukelman for her skilful assistance during the preparation of the manuscript.

References

1. Armstrong AS, Mathias JR, DeYoung MI, *et al.* Strain differentiation of *Neisseria gonorrhoeae* by reverse passive hemagglutination. *Infect Immun* 1979;**24**:51-8.
2. Reyn A, Bentzon MW. Comparison of a selective and a non-selective medium in the diagnosis of gonorrhoea to ascertain the sensitivity of *Neisseria gonorrhoeae* to vancomycin. *Br J Vener Dis* 1972;**48**:363-8.
3. Taylor E, Phillips I. Assessment of a selective medium for the isolation of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1979;**55**: 183-5.
4. Takeguchi MM, Weetall HH, Smith DK, *et al.* Enzymatic detection of *Neisseria gonorrhoeae*. *Br J Vener Dis* 1980;**56**: 304-7.

Br J Vener Dis 1982;**58**:363-5

Induction in gonococci of phenotypic resistance to killing by human serum by human genital secretions

P M V MARTIN,* P V PATEL,† N J PARSONS,† AND H SMITH†

*From the *Unité d'Ecologie Bactérienne, Institut Pasteur, Paris, France, and the †Department of Microbiology, University of Birmingham, Birmingham*

SUMMARY Cervical secretions and seminal plasmas from uninfected clinically healthy persons and vaginal exudates from patients with infections other than gonorrhoea converted gonococci susceptible to killing by fresh human serum to resistance after three hours' incubation at 37°C. The inducing factors present in the genital secretions explain the serum resistance of gonococci from patients with acute urethritis. This serum resistance is lost on subculture in laboratory media but could play an important role in vivo in the survival of gonococci in the initial stages of urogenital infection when serum factors are liberated into the local infection site during inflammation.

Introduction

Gonococci obtained from infected patients are resistant to killing by human serum. This resistance is accepted as important in the pathogenesis of gonorrhoea in relation to survival in the bloodstream in disseminated infection.[1] Less attention has been paid to its possible role in local infections of the mucous membranes and subepithelial tissues, where it could be equally important in promoting survival against serum factors which will certainly be liberated into the area by the intense inflammatory response which is evoked by gonococcal infection. A fact sometimes forgotten in the universal acceptance of the serum resistance of strains from disseminated infections[2] is that gonococcal strains isolated from urethral pus and examined directly without subculture are also resistant to killing by human serum.[3] The latter resistance is, however, different from the former. Whereas most strains from disseminated infections retain their resistance to human serum on subculture many of the isolates from acute urethritis lose their resistance in laboratory cultures.[3] We have been interested in this unstable type of resistance—that is, what host factors induce the resistance in vivo that are absent from artificial media. Firstly, using infection of subcutaneously implanted plastic chambers in guinea pigs as a convenient source of serum (human)-resistant gonococci grown in vivo, it was shown that resistance was phenotypically determined[4] and induced by factors in guinea pig serum, one of which was of low molecular weight.[5 6] Attention then turned to human inducing factors which were found in about 25% of human sera and in both high and low molecular weight fractions.[7] In relation to the induction or maintenance of serum resistance in gonococci at the beginning and early stages of mucosal infection, female and male genital secretions have now been examined for resistance-inducing activity.

Materials and methods

GENITAL SECRETIONS

At Birmingham cervical scrapings were obtained from uninfected, clinically healthy subjects by courtesy of Drs R Condie, K K Chan, R Patel, and H Leung (St Chad's Hospital). Scrapings were collected in saline (5-10 ml) and kept at −20°C until required for tests. After being thawed at 37°C, the samples were sonicated (Rapidis Ultrasonic SP-957, Ultrasonic Ltd, Shipley, Yorks; 50 watts, 20 kHz; four bursts of 30 seconds at room temperature) and centrifuged (2500 × *g* for 15 minutes at room temperature). The supernatant fluid was separated, filtered through a Millipore membrane (0·22 μm pore size), and tested for resistance-inducing activity. In the first instance nine samples were bulked for testing and then subsequent samples were tested individually.

At Birmingham seminal plasma was also collected from uninfected, clinically healthy subjects by

Address for reprints: Dr P V Patel, Department of Microbiology, University of Birmingham, South-west Campus, P O Box 363, Birmingham, B15 2TT

Accepted for publication 15 July 1982

courtesy of Mr C J Lodge (St Chad's Hospital) and Mr P J Adams (Selly Oak Hospital). Each sample (about 2 ml) was processed without dilution with saline. They were sonicated, centrifuged, and filtered through a Millipore membrane as described for the cervical secretions. Owing to the high viscosity only 0·1-0·5 ml of filtered material was available for testing for resistance-inducing activity.

In Paris vaginal exudates were collected by courtesy of Dr F Pean (International Students' Hospital) from 14 clinically infected patients without bacteriologically diagnosed gonorrhoea; the diagnosed infections were viral, one, bacterial other than gonococcal, four, fungal, two, and parasitic, one. All samples (0·2-4·5 ml) contained about 50% (v/v) cellular debris. After standing at 4°C for 3-4 hours the samples were diluted with an equal volume of defined medium (see below) at pH 7 ± 0·2. After vortex mixing and centrifuging (3000 × g for 10 minutes at room temperature) the supernatant fluid was separated, adjusted to pH 6·6 ± 0·2, heated for 40 minutes at 56°C, filtered through Millipore membrane (0·22-μm pore size) and stored at −20°C until tested for resistance-inducing activity.

SERUM-SENSITIVE *NEISSERIA GONORRHOEAE*

This was strain BS4 (agar) derived, stored, cultured, and viably counted as described.[5 7 8]

GENERATION OF RESISTANT ORGANISMS

The method was performed as described.[5 7] Briefly the medium (100 μl) consisted of a 1 : 1 mixture of a defined medium (DM)[5] and test sample (see above) at pH 6·6 ± 0·2 placed in the wells of Microtiter plates (Flow Laboratories, Irvine, UK). Serum-sensitive gonococci ($0{\cdot}5\text{-}2 \times 10^5$ ml^{-1} in 10 μl of DM) were added and the mixtures incubated for three hours at 37°C. Estimates of gonococcal multiplication were made by plating out duplicate samples (10 μl) of the mixture before and after incubation.

MEASUREMENT OF RESISTANCE TO KILLING BY FRESH HUMAN SERUM

This technique has been described.[5] The results were expressed as the number of colony forming units (cfu) recovered after incubation of a sample (4 μl) of the above suspension of gonococci for 40 minutes at 37°C with fresh human serum (FHS; 10 μl + 30 μl DM) quoted as a percentage of the cfu obtained when heat-inactivated (56°C; 60 min) serum was used. The following controls were included.

In each experiment a sample of heat-inactivated human serum known to have negligible resistance-inducing activity was included in the system for generation of resistant organisms; the gonococci so produced were shown to be completely susceptible (less than 1% survivors) to killing by fresh human serum in the test system described. Also, in the work with cervical secretions and seminal plasmas samples of small molecular weight fractions from guinea pig serum[5 6] known to be active or inactive in inducing resistance were included as additional controls. Finally, the absence of appreciable amounts of inhibitors of bactericidal activity (for example, anticomplimentary materials) in the genital secretions was checked by testing the bactericidal activity of FHS against susceptible BS4 (agar) organisms with and without the amount of genital secretion which would have been carried over from the generation of resistant organisms into the bactericidal test.

All samples of genital secretions were tested 2-3 times and typical results are quoted below.

Results

RESISTANCE INDUCTION

By cervical secretions from uninfected clinically healthy women

A bulked sample of secretions from nine people consistently converted 50-60% of the susceptible gonococci to resistance. Eleven individual samples of secretions converted 100%, 61%, 60%, 60%, 51%, 48%, 46%, 30%, 26%, 20%, and <10% of susceptible gonococci to resistance. In all these experiments the number of viable gonococci present at the end of the three-hour conversion period was 80-130% of those present initially.

By seminal plasmas from uninfected clinically healthy men

In preliminary tests two seminal plasmas both converted 100% of the susceptible gonococci to resistance. In subsequent tests 10 different seminal plasmas converted 100%, 97%, 92%, 92%, 91%, 91%, 90%, 71%, 60%, and 13% of susceptible gonococci to resistance. Some seminal plasmas had a slight ability to inhibit the bactericidal activity of human serum (see above). With these samples, to make sure that any carry-over of plasma from the conversion mixture did not interfere with the subsequent test for resistance the gonococci were centrifuged (3500 × g for 10 minutes) at the end of the three-hour conversion period and washed twice with 500 μl of DM containing 0·1% bovine serum albumin before subsequent testing. The results of the resistance tests on these washed gonococci were almost identical with those in which the gonococci were not washed. In all these experiments the number of viable gonococci present at the end of the three-hour conversion period was 70-200% of those present initially. Ten additional samples of seminal plasma showed some toxicity to gonococci when used undiluted in the conversion media but twofold-fourfold dilutions of all samples were not toxic and retained substantial resistance-inducing ability.

By vaginal exudates from patients with infections other than gonorrhoea

Two of the samples of vaginal secretions killed all gonococci in the three-hour incubation period probably owing to the presence of antibiotics from local treatment or to the toxic action of materials liberated from the phagocytes of the local inflammatory response. Of the remaining 12 samples, four showed distinct resistance-inducing activity converting 34%, 50%, 67%, and 100% of the susceptible gonococci to resistance in three hours at 37°C, two had low activity (conversion less than 20%), and the remainder appeared inactive. At the end of the three-hour conversion period for the active samples the number of viable gonococci was 110-230% of those present initially.

Discussion

Genital secretions from both women and men contain factor(s) which render gonococci resistant to killing by human serum, and the presence of these factors explains the serum resistance of gonococci examined directly from patients with acute urethritis.[3] The resistance-inducing activity of seminal plasma recalls the demonstration that addition of extract of prostate gland to laboratory media preserved the serum resistance of recent gonococcal isolates.[9] In this respect it is interesting that three of the 10 inducer-positive samples of seminal plasma (see results) came from subjects who had had vasectomies. Preliminary ultrafiltration experiments similar to those reported for guinea pig and human serum[6 7] indicated that the resistance inducing factor(s) in cervical secretions are present in similar low molecular-weight fractions.[6]

It is difficult to believe that the serum resistance of gonococci on and within genital epithelium does not play a role in survival in the initial stages of infection. While it is true that there is no direct evidence that the gonocidal factors in human serum are liberated into the local genital sites during inflammation this almost certainly happens, because there is good reason to believe that the lethal factors are natural antibody and complement which would, without doubt, be mobilised to the site of infection.

In the present study no attempt was made to assess the relative amounts of resistance-inducing factors in the different secretions examined and in the individual samples of each secretion. Clearly, the seminal plasmas were examined in a more concentrated form than the saline extracts of the cervical secretions and the DM-diluted vaginal secretions. In view of the probable importance of the inducing factors in the pathogenesis of gonorrhoea a more comprehensive and quantitative survey of genital secretions from various sources may be warranted. This would be easier to accomplish if the chemical nature of the inducer was known. Work to this end is in progress.

Finally, this study has underlined the fact that most gonococci whether they are causing local or disseminated infection are resistant in vivo to killing by human serum. Hence, the ability of some strains to cause disseminated infection must rely on pathogenic mechanisms in addition to being resistant to killing by human serum.

Our thanks are due to Dr E Boxall, of the East Birmingham Hospital, for checking that all samples of cervical secretions and seminal plasmas were free from hepatitis B virus.

References

1. Brooks GF, Gotschlich EC, Holmes KK, Sawyer WD, Young FE. *Immunobiology of Neisseria gonorrhoeae*. Washington DC: American Society for Microbiology, 1978.
2. Schoolnik GK, Buchanan TM, Holmes KK. Gonococci causing disseminated gonococcal infection are more resistant to the bactericidal action of normal human sera. *J Clin Invest* 1976;58:1163-73.
3. Ward ME, Watt PJ, Glynn AA. Gonococci in urethral exudate possess a virulence factor lost on subculture. *Nature* 1970;227:382-4.
4. Rittenberg SC, Penn CW, Parsons NJ, Veale DR, Smith H. Phenotypic changes in the resistance of *Neisseria gonorrhoeae* to killing by normal human serum. *J Gen Microbiol* 1977;103:69-75.
5. Veale DR, Penn CW, Smith H. Factors affecting the induction of phenotypically determined serum resistance of *Neisseria gonorrhoeae* grown in media containing serum or its diffusible components. *J Gen Microbiol* 1981;122:235-45.
6. Veale DR, Penn CW, Parsons NJ, Smith H. Preliminary studies of a factor in guinea pig serum which induces serum resistance in *Neisseria gonorrhoeae. Soc Gen Microbiol Quart* 1980;7:188.
7. Martin PMV, Patel PV, Parsons NJ, Smith H. Induction of phenotypically determined resistance of *Neisseria gonorrhoeae* to human serum by factors in human serum. *J Gen Microbiol* 1981;127:213-7.
8. Penn CW, Veale DR, Smith H. Selection from gonococci grown in vitro of a colony type with some virulence properties of organisms adapted in vivo. *J Gen Microbiol* 1977; 100:147-58.
9. Watt P, Glynn AA, Ward ME. Maintenance of virulent gonococci in laboratory culture. *Nature* 1972;236:186-7.

Br J Vener Dis 1982;**58**:366-9

Chlamydial genital infection in Ibadan, Nigeria

A seroepidemiological survey

S DAROUGAR,* T FORSEY,* A O OSOBA,† R J DINES,* B ADELUSI,‡ AND G O COKER†

*From the *Subdepartment of Virology, Institute of Ophthalmology, London; and the Departments of †Medical Microbiology and ‡Obstetrics and Gynaecology, University College Hospital, Ibadan, Nigeria*

SUMMARY Sera from patients attending a sexually transmitted diseases (STD) clinic, a family planning clinic, and an antenatal clinic in Ibadan, Nigeria, as well as from male blood donors from the same area were tested for the presence of type specific antichlamydial antibodies using a modified micro-immunofluorescence test.

Among men and women attending the STD clinic the exposure rates to *Chlamydia trachomatis* serotypes D to K (genital pathogens) were 18·7% and 26·7% respectively. Antibody titres suggesting active disease in these men and women were found in 11·8% and 22·7% respectively. The highest rate of exposure (35%) was among women attending the family planning clinic; of these women 25% had antibody suggesting active disease. Titres of IgG antibody in this study were similar to those found among men and women with chlamydial genital infections in the United Kingdom. Antibodies to serotypes D to K were also detected in 10·3% of women attending an antenatal clinic and in 9·9% of male blood donors. The prevalence of antibodies to *C trachomatis* serotypes A to C and lymphogranuloma venereum serotypes was low.

These results suggest that the prevalence of chlamydial genital infections in Ibadan, both among STD patients and especially among those individuals not seeking treatment (family planning and antenatal clinic patients), is high. Since serious sequelae can follow chlamydial genital infections it is imperative to carry out further investigations in this area.

Introduction

In the developed countries of the West, the prevalence of non-gonococcal genital infections (NGGI) is higher than that of gonococcal genital infection.[1 2] Although no accurate figures are available for most of the developing countries it has been estimated that NGGI are as prevalent in these areas as in the West.[3 4]

Studies have shown that in developed countries *Chlamydia trachomatis* is responsible for up to 68% of cases of non-gonococcal urethritis in men[5] and has been isolated from the cervix of up to 31% of women attending sexually transmitted diseases (STD) clinics.[6] In this study we determined the prevalence of chlamydial genital infections in various groups in Ibadan, Nigeria, by detecting type-specific antichlamydial antibodies using a modified micro-immunofluorescence (micro-IF) test.

Patients and methods

Included in this study were: 187 men and 75 women who attended consecutively the sexually transmitted diseases (STD) clinic, Ibadan; 160 women who attended consecutively a family planning clinic, 155 women attending consecutively an antenatal clinic for routine advice and examination; and 101 unselected male blood donors from the same town. Patients in the last three groups had no symptoms of overt genital disease.

Blood was taken from all individuals by venepuncture; the serum was separated and stored at −20°C. Sera were shipped to the laboratory in London in the frozen state and stored at −20°C until tested.

Address for reprints: Professor S Darougar, Subdepartment of Virology, Institute of Ophthalmology, Judd Street, London WC1H 9QS

Accepted for publication 28 June 1982

Sera were tested for the presence of type-specific antichlamydial antibodies using pools of purified chlamydial antigens in a modified micro-IF test.[7] Sera were examined at a starting dilution of 1/16 for antichlamydial IgG and of 1/8 for antichlamydial IgM.

Results

The distribution of antibodies to chlamydial serotypes in the population examined are shown in table I. Antibodies to *C trachomatis* serotypes D to K (genital pathogens) were found in 35 of 187 (18·7%) men and in 20 of 75 (26·7%) women attending the STD clinic. In women attending the family planning and antenatal clinics antibodies to serotypes D to K were found in 56 of 160 (35%) and 16 of 155 (10·3%) respectively. Among 101 male blood donors these antibodies were detected in 10 (9·9%).

The prevalence of IgG and IgM antibodies specific for *C trachomatis* serotypes D to K is shown in table II. Antibodies suggesting active chlamydial disease were found in 22 (11·8%) men and 17 (22·7%) women attending the STD clinic, in 40 (25%) and six (3·9%) women attending family planning and antenatal clinics respectively, and in five (5%) male blood donors. The distribution of titres of IgG antibody to serotypes D to K is shown in table III.

Antibodies to *C trachomatis* serotypes A to C (trachoma types) were found in a total of 22 of 678 (3·2%) sera from all groups, and antibodies to lymphogranuloma venereum (LGV) types were detected in only two (0·3%) (both from men). Antibodies against *Chlamydia psittaci* serotypes were not detected.

Discussion

The patients attending the STD clinic, Ibadan, presented with a variety of genital diseases. The diagnoses made in these patients included syphilis, gonorrhoea, non-gonococcal urethritis, cervicitis,

TABLE I *Distribution of antibodies to chlamydial serotypes in Ibadan, Nigeria*

Patient group (sex)	*No of patients*	*Total No (%) with antibodies*	*No (%) with antibibodies to C trachomatis serotypes**		
			A-C	*D-K*	*LGV 1-3*
STD clinic (M)	187	40 (21·4)	4 (2·1)	35 (18·7)	1 (0·5)
STD clinic (F)	75	21 (28·0)	1 (1·3)	20 (26·7)	0
Family planning clinic (F)	160	64 (40·0)	8 (5·0)	56 (35·0)	0
Antenatal clinic (F)	155	18 (11·6)	2 (1·3)	16 (10·3)	0
Blood donors (M)	101	18 (17·8)	7 (6·9)	10 (9·9)	1 (1·0)
Total	678	161 (23·8)	22 (3·2)	137 (20·2)	2 (0·3).

*All sera negative for *C psittaci*

TABLE II *Prevalence of antibodies to C trachomatis serotypes D to K in Ibadan, Nigeria*

Patient group (sex)	*No*	*No (%) with IgG*	*No (%) with IgM*	*No (%) with antibodies suggesting active infection**
STD clinic (M)	187	35 (18·7)	7 (3·7)	22 (11·8)
STD clinic (F)	75	20 (26·7)	6 (8·0)	17 (22·7)
Family planning clinic (F)	160	56 (35·0)	24 (15·0)	40 (25·0)
Antenatal clinic (F)	155	13 (8·4)	3 (1·9)	6 (3·9)
Blood donors (M)	101	8 (7·9)	2 (2·0)	5 (5·0)
Total	678	132 (19·5)	42 (6·2)	90 (13·3)

*Men IgG ≥32 or IgM
Women IgG ≥64 or IgM

TABLE III *Distribution of titres of IgG to C trachomatis serotypes D to K in Ibadan, Nigeria*

		Titres							
Patient group (sex)	*No*	<16	16	32	64	128	256	>256	GMT*
STD clinic (M)	187	152	17	2	10	3	3		1/37
STD clinic (F)	75	55	2	5	6	2	5		1/71
Family planning clinic (F)	160	104	8	15	12	8	7	6	1/72
Antenatal clinic (F)	155	142	7	3	2		1		1/29
Blood donors (M)	101	93	5	3					1/21

*Geometric mean titre of positive sera

epididymitis, vaginitis, candidosis, trichomoniasis, and genital herpes. Some patients presented with vague genital symptoms and others as contacts of patients with STDs. Among 187 of these men and 75 of these women antibodies to chlamydial genital pathogens (*C trachomatis* serotypes D to K)[8] were found in 18·7% and 26·7% respectively. To the best of our knowledge comparable findings are not available from similar groups elsewhere in the world. Among a group of 146 men, however, with either post-gonococcal or non-gonococcal urethritis in the United Kingdom antibodies to serotypes D to K were found in 25% using the same micro-IF test.[9] Among 272 women attending a VD clinic in London with various STDs, these antibodies were detected by the micro-IF test in 43%.[10] Therefore, considering the nature of the patient groups studied the prevalence of chlamydial genital infections in the STD clinic in Ibadan is not dissimilar to that found in the United Kingdom. The titres of antichlamydial IgG in men (geometric mean titre (GMT) 1/37) and women (GMT 1/71) in Ibadan were also similar to those found in the United Kingdom among men with chlamydial urethritis[9] and women with chlamydial cervicitis.[10]

When serum IgG titres of ≥32 in men and ≥64 in women or the presence of antichlamydial IgM are considered to suggest active chlamydial infection,[9 10] 11·8% of men and 22·7% of women from this clinic in Ibadan appeared to have active chlamydial genital infections at the time of examination. In a previous study from the same clinic, 26% of all patients had NGGI.[11] Our results suggest that most of these infections may be due to chlamydiae and that women are the main resevoir of infection.

Perhaps the most striking finding of this study is that among 160 women attending a family clinic in Ibadan antibodies to *C trachomatis* serotypes D to K were detected in 35%. Of these 25% had antibody titres suggesting active infection. This prevalence is higher than would be expected in the United Kingdom or USA, where 3% and 11% of women attending family planning clinics had chlamydial genital infections.[6 12] The titres of IgG among these women in Ibadan (GMT 1/72) were similar to those in the women attending the STD clinic and to those in women in the United Kingdom with chlamydial genital infections.[10]

Of 155 women presenting for routine antenatal examinations antibodies to serotypes D to K were found in the sera of 10·3%. Although the IgG was generally of a low titre (GMT 1/29), 3·9% of these women had values suggesting active infection. Similarly, among 101 male blood donors, 9·9% had these antibodies (GMT 1/21) and 5% had evidence of active infection. By comparison, using the same serological test in the United Kingdom we found IgG antibody to *C trachomatis* serotypes D to K in only 3% of female and less than 1% of male blood donors.[13] Antichlamydial IgM could not be detected in sera from these blood donors in the UK.

Only two patients, both men, had antibodies to LGV serotypes, indicating a very low prevalence of exposure (less than 1%) in this population. This finding agrees with a previous study in Nigeria, where among patients with STD the prevalence of LGV was also found to be low at 2·5%.[3] Few patients (3%) in Ibadan had antibodies specific for *C trachomatis* serotypes A to C, the types commonly associated with hyperendemic trachoma.[14]

In Nigeria tetracycline is widely available and commonly abused through self-medication for minor ailments. Although chlamydiae are sensitive to this antibiotic the seroreactivity to *C trachomatis* serotypes D to K was high among the patients studied. Our results from Ibadan suggest that chlamydial genital infections occur among patients attending an STD clinic with a prevalence similar to that found in developed countries. These infections are also prevalent in Ibadan among other groups, in which the disease was mild or asymptomatic. Studies have shown that such conditions as epididymitis, pelvic inflammatory disease, and perihepatitis can result as sequelae of chlamydial genital infections.[15-17] In Africa pelvic inflammatory disease and infertility pose a major problem[2]; it is therefore imperative that the role of chlamydiae in these diseases receives further investigation.

This study was supported in part by the World Health Organisation, Ibadan.

References

1. Schachter J, Hanna L, Hill EC, *et al*. Are chlamydial infections the most prevalent venereal disease? *JAMA* 1975;**231**:1252-5.
2. Catterall RD. Biological effects of sexual freedom. *Lancet* 1981;i:315-9.
3. Osoba AO. Sexually transmitted diseases in tropical Africa. *Br J Vener Dis* 1981;**57**:89-94.
4. Rotimi VO, Somorin AD. Sexually transmitted diseases in clinic patients in Lagos. *Br J Vener Dis* 1980;**56**:54-6.
5. Alani MD, Darougar S, MacD Burns DC, Thin RN, Dunn H. Isolation of *Chlamydia trachomatis* from the male urethra *Br J Vener Dis* 1977;**53**:88-92.
6. Hilton AL, Richmond SJ, Milne JD, Hindley F, Clark SKR. *Chlamydia A* in the female genital tract. *Br J Vener Dis* 1974;**50**:1-10.
7. Treharne JD, Darougar S, Jones BR. Modification of the micro-immunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977;**30**:510-7.
8. Dunlop EMC, Darougar S, Treharne JD. Epidemiology of infection by serotypes D to K of *Chlamydia trachomatis*. *Br J Vener Dis* 1980;**56**:163-8.
9. Treharne JD, Dines RJ, Darougar S. Serological responses to chlamydial ocular and genital infections in the United Kingdom and Middle East. In: Hobson D, Holmes KK, eds. *Non-gonococcal urethritis and related infections*. Washington DC: American Society for Microbiology, 1977; 249-58.

10. Treharne JD, Darougar S, Simmons PD, Thin RN. Rapid diagnosis of chlamydial infection of the cervix. *Br J Vener Dis* 1978;**54**:403-8.
11. Sogbetun AO, Alausa KO, Osoba AO. Sexually transmitted diseases in Ibadan, Nigeria. *Br J Vener Dis* 1977;**53**:155-60.
12. Schachter J. Chlamydial infections. *N Engl J Med* 1978;**298**:490-5.
13. Darougar S, Forsey T, Brewerton DA, Roger KLI. Prevalence of antichlamydial antibody in London blood donors. *Br J Vener Dis* 1980:**56**:404-7.
14. Jones BR. Prevention of blindness from trachoma. *Trans Ophthalmol Soc UK* 1975;**95**:16-33
15. Harnish JP, Berger RE, Alexander ER, Manda G, Holmes KK. Aetiology of acute epididymitis. *Lancet* 1977;**i**:819-21.
16. Mårdh P-A, Ripa KT, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977;**296**:1377-9.
17. Müller-Schoop JW, Wang S-P, Munzinger J, Schlapfer HU, Knoblauch M, Ammann RW. *Chlamydia trachomatis* as a possible cause of peritonitis and perihepatitis in young women. *Br Med J* 1978;**i**:1022-4.

Br J Vener Dis 1982;**58**:370-3

Chlamydial genital infection in Addis Ababa, Ethiopia

A seroepidemiological survey

T FORSEY,* S DAROUGAR,* R J DINES,* D J M WRIGHT,† AND P S FRIEDMANN‡

*From the *Subdepartment of Virology, Institute of Ophthalmology, London; the †Department of Microbiology, Charing Cross Hospital, London; and the ‡Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne*

SUMMARY A seroepidemiological survey was undertaken in Addis Ababa to assess the prevalence of chlamydial genital infections among patients attending a sexually transmitted diseases (STD) clinic and patients with no overt genital symptoms. In the STD clinic patients antibodies to *Chlamydia trachomatis* serotypes D to K (genital types) were detected in 68 of 210 (32·4%) men and in 72 of 159 (45·3%) women, a rate of exposure as high or higher than that found in Europe. Serological evidence of active chlamydial infection was present in 26·7% of men and 28·9% of women. Women were at risk of contracting STD, including chlamydial infections, at the age of 14 years or earlier. The titres of antichlamydial IgG were extremely high in some patients attending the STD clinic, with titres of between 1/512 and 1/8192 in 9·5% of men and 13·2% of women. This suggests that some patients had severe or disseminated chlamydial disease. The prevalence of exposure to chlamydial genital infections among 148 patients with no overt genital disease was 14·2%, which is significantly higher than that found in the United Kingdom.

Among the total of 517 patients tested the prevalence of exposure to trachoma, lymphogranuloma venereum, and *Chlamydia psittaci* agents was very low.

Introduction

Recent studies in Ethiopia suggest that sexually transmitted diseases (STD) are common[1] and that social factors, such as informal marriage contracts and extensive prostitution, play an important role in the widespread dissemination of these diseases.[2]

In developed countries chlamydial genital infections and their complications, such as epididymitis[3] and salpingitis,[4] are well recognised. The prevalence of these infecions in most developing countries, however, is not known, although a recent serological survey in Nigeria[5] indicated that chlamydial genital infections were as common there as in Europe.

We present the results of a seroepidemiological survey conducted in Addis Ababa of chlamydial genital infections among patients attending an STD clinic and among patients admitted to hospital for other reasons.

Patients and methods

All patients came from Addis Ababa and no special basis of selection was used. They included: 210 men (mean age 28±12 years) and 159 women (mean age 27±13 years), attending as new patients at the Venereal Disease Centre and presenting with a variety of genitourinary symptoms; 111 male and female inmates of the Amanuel Mental Hospital (mean age 35±15 years); and 37 male and female patients with leprosy from the Alert Hospital (age unknown).

Serum was collected from each patient and tested for the presence of type-specific IgG and IgM antichlamydial antibodies at starting dilutions of 1/16 and 1/8 respectively. Pools of chlamydial antigens were derived from trachoma, oculogenital,

Address for reprints: Mr T Forsey, Subdepartment of Virology, Institute of Ophthalmology, Judd Street, London WC1H 9QS

Accepted for publication 28 June 1980

and lymphogranuloma venereum (LGV) serotypes of *C trachomatis* and selected isolates of *C psittaci.* These antigens were incorporated in a modified micro-immunofluorescence (micro-IF) test,[5a] which was used to test all sera. Serum antibody titres suggesting active chlamydial genital infection were taken as IgG ≥32 or IgM ≥8 for men and IgG ≥64 and IgM ≥8 for women.[6,7]

Results

The distribution of antibodies against the various chlamydial serotypes is shown in table I. Antibodies specific for *C trachomatis* serotypes D to K (genital types[8]) were found in 161 of 517 (31·1%) patients tested. The prevalence of IgG and IgM antibodies specific for *C trachomatis* serotypes D to K is shown in table II.

Active chlamydial genital infection (IgG titres ≥32 or IgM titres ≥8 in men and IgG titres ≥64 or IgM titres ≥8 in women) was evident serologically in 56 (26·7%) men and 46 (28·9%) women from the VD centre.

The titres of antibodies to serotypes D to K are shown in tables III and IV. Extremely high titres of IgG were found in some of the patients attending the VD centre; 20 of 210 (9·5%) men and 21 of 159 (13·2%) women had IgG titres between 512 and 8192. The distribution of these antibody results among various age groups in the VD centre population is shown in table V.

Of the 517 individuals tested, antibodies to *C trachomatis* serotypes A to C (trachoma types[9]) were found in six (1·2%), to LGV serotypes in two (0·4%), and to *C psittaci* in only one (0·2%) (table I).

TABLE I *Distribution of antibodies to chlamydial serotypes in Addis Ababa, Ethiopia*

Patient group (sex)	*No of patients*	*Total No (%) with antibodies*	*No (%) with antibodies to:*			
			C trachomatis serotypes			*C psittaci*
			A-C	*D-K*	*LGV 1-3*	
VD centre (M)	210	72 (34·3)	3 (1·4)	68 (32·4)	1 (0·5)	0
VD centre (F)	159	75 (47·2)	3 (1·9)	72 (45·3)	0	0
Amanuel Mental Hospital	111	15 (13·5)	0	13 (11·7)	1 (0·9)	1 (0·9)
Alert Leprosy Hospital	37	8 (21·6)	0	8 (21·6)	0	0
Total	517	170 (32·9)	6 (1·2)	161 (31·1)	2 (0·4)	1 (0·2)

TABLE II *Prevalence of serum antibodies to C trachomatis serotypes D to K in Addis Ababa, Ethiopia*

Patient group (sex)	*No of patients*	*No (%) IgG-positive**	*No (%) IgM-positive†*	*Total No (%) positive*
VD centre (M)	210	67 (31·9)	37 (17·6)	68 (32·4)
VD centre (F)	159	71 (44·7)	42 (26·4)	72 (45·3)
Amanuel Mental Hospital	111	12 (10·8)	1 (0·9)	13 (11·7)
Alert Leprosy Hospital	37	8 (21·6)	0	8 (21·6)
Total	517	158 (30·6)	80 (15·5)	161 (31·1)

*IgG-positive = titre ≥16
†IgM-positive = titre ≥8

TABLE III *Distribution of titres of serum IgG antibody to C trachomatis serotypes D to K in patients in Addis Ababa, Ethiopia*

Patient group (sex)	*No of patients*	*Serum IgG antibody reciprocal titre*							
		<16	*16*	*32*	*64*	*128*	*256*	*512-8192*	*GMT**
VD centre (M)	210	143	12	9	9	7	10	20	1/138
VD centre (F)	159	88	10	15	6	8	11	21	1/193
Amanuel Mental Hospital	111	99	9	1	2				1/38
Alert Leprosy Hospital	37	29	2	2	1	3			1/49

*Geometric mean titre of sera containing IgG antibody at titre of ≥16

LIBRARY

TABLE IV *Distribution of IgM titres to C trachomatis serotypes D to K in Addis Ababa; Ethiopia*

Patient group (sex)	*No of patients*	*Serum IgM antibody reciprocal titre*						
		<8	*8*	*16*	*32*	*64*	*128*	*GMT**
VD centre (M)	210	173	3	6	14	5	9	1/23
VD centre (F)	159	117	10	3	11	9	9	1/31
Amanuel Mental Hospital	111	110	1					1/8
Alert Leprosy Hospital	37	37						

*Geometric mean titre of sera containing IgM antibody at a titre of ⩾8

TABLE V *Distribution of antibodies to C trachomatis serotypes D to K in VD Centre, Addis Ababa, related to age of patients*

Age (years)	*No of patients**	*No antibody-positive (%)*
Men:		
⩽14	1	0
15-29	149	45 (30·2)
30-49	48	20 (41·7)
⩾50	8	1 (12·5)
Women:		
⩽14	9	2 (22·2)
15-29	91	42 (46·2)
30-49	37	21 (56·8)
⩾50	17	5 (29·4)

*Ages known in 206 men and 154 women

Discussion

Among unselected patients attending the VD centre, Addis Ababa, the prevalence of exposure to chlamydial genital infections, as indicated by the presence of antibodies to *C trachomatis* serotypes D to K, was 32·4% in men and 45·3% in women. Studies using the same serological test have detected antichlamydial antibodies in 32% of selected men with non-gonococcal urethritis[6] and in 43% of women attending an STD clinic[7] in the United Kingdom and in up to 39% of South African women attending an STD clinic.[10] In a recent study in Nigeria these antibodies were detected in 19% and 27% of men and women respectively attending an STD clinic.[5] Our results suggest that the rate of exposure to chlamydial genital infections is as high or higher in Addis Ababa than elsewhere.

Serum antibody titres suggesting active chlamydial infection were present in 26·7% of men and 28·9% of women attending the VD centre. In the Nigerian STD clinic population 12% of men and 23% of women had antibodies at these titres.[5]

The titres of IgG and IgM antibodies to serotypes D to K among patients attending the STD clinic, Addis Ababa, were much higher than those found in the United Kingdom or Nigeria. The geometric mean titre (GMT) of IgG among these men from Addis Ababa was 1/138, and 9·5% of patients had titres between 512 and 8192. Among the women the GMT was 1/193 and 13·2% had titres at these extremely high levels. Using the same serological test in the United Kingdom men with chlamydial urethritis had serum IgG titres of around 32[5a 6] and women with chlamydial cervicitis titres of around 64.[5a 7] We also found similar titres in patients attending an STD clinic in Nigeria (GMT 1/37 in men and 1/71 in women). The titres of antibodies to serotypes D to K in these Ethiopian men constituted the highest titres yet detected by us in any group of men. The titres in the women were similar to those found by us in women in the United Kingdom with pelvic inflammatory disease (GMT 1/98 and 1/160)[12 13] and perihepatitis (GMT 1/149).[14] These patients from Addis Ababa thus may have more severe or disseminated chlamydial genital disease than is usually seen in STD patients either from the United Kingdom or Nigeria.

The highest proportion of patients attending the VD centre who had antibodies to serotypes D to K were found among those aged 15 to 49 years, the age range of greatest sexual activity. Although the numbers were small more women than men were exposed to chlamydial genital infections at 14 years or under and at 50 years or over. Similarly more women than men of these ages actually attended the STD clinic with a complaint. Thus, in this sexually active female population there is a risk of contracting STD at 14 years of age or earlier. This may be a reflection of the widespread prostitution in Addis Ababa.[2]

Patients from the Amanuel and Alert hospitals were not accurately matched as controls for patients attending the VD centre nor were they representative of the general population of Addis Ababa; however, they were individuals without overt signs of genital disease. Among these groups the overall prevalence of exposure to chlamydial genital infection was 14·2%. One (0·7%) patient had antichlamydial IgM and six (4·1%) IgG at titres of ⩾64. This level of exposure is appreciably higher than that found in the United Kingdom, where, for example, among male and female blood donors a prevalence of only 2% was found using the same laboratory test.[15]

Antibodies to *C trachomatis* serotypes A to C (trachoma types) were detected in six of 517 (1·2%) sera tested. The prevalence of LGV and *C psittaci* infections were also low at 0·4% and 0·2% respectively. The low prevalence of LGV is similar to our findings in Nigeria,[5] where these antibodies were detected in 0·3% of the population studied.

Thus, the prevalence of chlamydial genital infections is high in Addis Ababa. This may reflect particular social conditions and the lack of facilities for diagnosis and treatment of STD in Ethiopia.[2]

References

1. Plorde DS. Sexually transmitted diseases in Ethiopia. *Br J Vener Dis* 1981;**57**:357-62.
2. Friedman PS, Wright DJM. Observations on syphilis in Addis Ababa. I General considerations. *Br J Vener Dis* 1977;**53**:273-5.
3. Harnisch JP, Berger RE, Alexander ER, Manda G, Holmes KK. Aetiology of acute epididymitis. *Lancet* 1977;**i**:819-21.
4. Mårdh P-A, Ripa KT, Svensson L, Weström L. *Chlamydia trachomatis* infection in patients with acute salpingitis. *N Engl J Med* 1977;**296**:1377-9.
5. Darougar S, Forsey T, Osoba AO, Dines RJ, Adelusi B, Coker GO. Chlamydial genital infection in Ibadan, Nigeria: a seroepidemiological survey. *Br J Vener Dis* 1982;**58**:366-9.

5a. Treharne JD, Darougar S, Jones BR. Modification of the microimmunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977;**30**:510-7.

6. Treharne JD, Dines RJ, Darougar S. Serological responses to chlamydial ocular and genital infections in the United Kingdom and Middle East. In: Hobson D, Holmes KK, eds. *Non-gonococcal urethritis and related infections.* Washington DC: American Society for Microbiology, 1977;249-8.
7. Treharne JD, Darougar S, Simmons PD, Thin RN. Rapid diagnosis of chlamydial infection of the cervix. *Br J Vener Dis* 1978;**54**:403-8.
8. Dunlop EMC, Darougar S, Treharne JD. Epidemiology of infection by serotypes D to K of *Chlamydia trachomatis. Br J Vener Dis* 1980;**56**:163-8.
9. Jones BR. Prevention of blindness from trachoma. *Trans Ophthalmol Soc UK* 1975;**95**:16-33.
10. Ballard RC, Fehler HG, Duncan MO, van der Wat IJ. Urethritis and associated infections in Johannesburg. The role of *Chlamydia trachomatis. South African Journal of Sexually Transmitted Diseases* 1981;**1**:24-6.
12. Simmons PD, Forsey T, Thin RN, Treharne JD, Darougar S, Langley F, Pandhi RK. Antichlamydial antibodies in pelvic inflammatory disease. *Br J Vener Dis* 1979;**55**:419-21.
13. Treharne JD, Ripa KT, Mårdh P-A, Svensson L, Weström L, Darougar S. Antibodies to *Chlamydia trachomatis* in acute salpingitis. *Br J Vener Dis* 1979;**55**:26-9.
14. Darougar S, Forsey T, Wood JJ, Bolton JP, Alan A. *Chlamydia* and the Curtis-Fitz Hugh syndrome. *Br J Vener Dis* 1981;**57**:391-4.
15. Darougar S, Forsey T, Brewerton DA, Rogers KLI. Prevalence of antichlamydial antibody in London blood donors. *Br J Vener Dis* 1980;**56**:404-7.

Br J Vener Dis 1982;**58**:374-6

Chlamydial urethral infection in Teheran

A study of male patients attending an STD clinic

S DAROUGAR,* BARRIE R JONES,* L CORNELL,* J D TREHARNE,*
R ST C DWYER,* AND B ARAMESH†

*From the *Subdepartment of Virology, Institute of Ophthalmology, London; and the †School of Public Health, Teheran, Iran*

SUMMARY The prevalence of chlamydial infection of the urethra was studied in 172 consecutive male patients attending a sexually transmitted disease clinic in Teheran. *Chlamydia trachomatis* was isolated in 8·8% of the patients with a valid culture result. Of the five isolates serotyped, two were serotype E and three were serotypes G, H, and K. Type-specific antibodies against *C trachomatis* serotypes D to K were found in 16% of patients, and IgM, indicating current infection, was detected in 12%. Type-specific antibodies against serotypes A to C (trachoma agent) were detected in 5·4%.

The low chlamydial isolation rate may have been due to the inclusion of a large number of patients with a mild or trivial urethritis or a history of previous treatment with antichlamydial drugs.

The results indicate that in Iran where trachoma is still endemic, chlamydial infection of the urethra does occur in the urban population and is caused by serotypes D to K.

Introduction

In the developing countries of the Middle East very little is known about the prevalence and patterns of sexually transmitted diseases (STD).[1] In particular, there is a lack of information about non-gonococcal urethritis (NGU) and chlamydial genital infection.

During our studies of trachoma in rural populations of northern and southern Iran we found that trachoma caused by *Chlamydia trachomatis* serotypes A, B, and C was still endemic in the villages.[2] In these communities we found no evidence of chlamydial genital infection or associated ocular infection caused by *C trachomatis* serotypes D to K (Darougar *et al*, unpublished data). We were told however, that NGU was common in the urban population of Iran.

The present study was undertaken to investigate the prevalence of chlamydial genital infections and serotypes among men attending an STD clinic in Teheran.

Patients and methods

One hundred and seventy-two consecutive male patients who attended the Nejat STD clinic, Teheran, and who gave a history of urethral discharge, dysuria, or frequency of micturition were studied.

Urethral specimens were collected using a cottonwool swab.[3] Specimens were stored in 2SP transport medium containing antibiotics[4] and 3% fetal calf serum. They were transferred within one hour of collection into a liquid nitrogen refrigerator (−180°C) until they reached the laboratory in London where they were stored in a refrigerator at −70°C. Methods of culture in irradiated McCoy cells and the identification of isolates have been described.[5]

Blood was taken by venepuncture; serum was separated, stored at −20°C, and transferred within three days at an ambient temperature to London, where they were stored at −20°C. All sera were absorbed with equal volumes of 40% (w/v) normal yolk-sac for 60 minutes and were then titrated at a starting dilution of 1/16 for IgG and 1/8 for IgM. A modified microimmunofluorescence (micro-IF) test[6] was used to detect type-specific antichlamydial IgG and IgM in sera. In the present study all sera were titrated against four pooled antigens; pool 1, *C trachomatis* serotypes A, B, and C; pool 2, *C trachomatis* serotypes D, E, F, G, H, I, and K; pool 3, *C trachomatis* LGV 1, 2 and 3; and pool 4, representative antigens from *C psittaci*.[6]

Isolates obtained in cell cultures were serotyped using a micro-IF test as described.[7]

Address for reprints: Professor S Darougar, Subdepartment of Virology, Institute of Ophthalmology, Judd Street, London WC1H 9QS

Accepted for publication 12 July 1982

Results

The ages of the 172 men ranged from 14 to 48 years with a mean age of 25 years. Of these men, 140 (81·4%) had been treated for confirmed or suspected urethritis within the previous year; 133 (77·3%) had been given tetracyclines, erythromycin, or a sulphonamide, and nine (5·2%) penicillin alone (table I).

TABLE I *History of previous treatment with antibiotics of a confirmed or suspected urethritis in 172 men*

History	*No (%) of patients treated with:* Penicillin	*No (%) of patients treated with:* Antichlamydial drugs*
Of antibiotic treatment:		
Within one month	6 (3·5)	48 (27·9)
Between one and three months	1 (0·6)	20 (11·6)
Between 3 and 12 months	2 (1·2)	63 (36·6)
Total	9 (5)	131 (76·2)

*Tetracyclines or erythromycin or sulphonamides with or without additional penicillin

Cultural and serological evidence of infection with *C trachomatis* was found in 39 (22·7%) patients. Of these, 31 (18%) were infected with serotypes D to K and eight (4·7%) with serotypes A to C. There was no evidence of infection with lymphogranuloma venereum agents or *C psittaci.*

The culture results were considered invalid in 47 (27·3%) of 172 patients because of gross bacterial and fungal contamination or poor cell monolayers. *C trachomatis* was isolated from 11 (8·8%) of the remaining 125 cases. Chlamydia were isolated from one (2·1%) of 48 patients who had received antimicrobial treatment within one month, from three (15%) of 20 men who had been treated between one and three months, and from six (9·5%) of 63 of those who were treated between three and 12 months before examination.

Type-specific antibodies against *C trachomatis* serotypes D to K were found in the sera of 23 (15·7%) of 147 patients tested. Antichlamydial IgM, indicating current infection, and IgG were detected in 17 (11·6%) and 14 (9·5%) sera respectively (table II). Of 48 patients who had been treated with antichlamydial drugs in the previous month, IgM reactive with *C trachomatis* was detected in 14 (29·2%) whereas *C trachomatis* was isolated in only one (2·1%) patient.

Type-specific antibodies against *C trachomatis* serotypes A to C were detected in eight (5·4%) patients (table II). Serological evidence of current or recent infection with these serotypes (antichlamydial IgG at a titre of ≥ 64 or IgM at a titre of ≥ 8[8]) was found in three patients; they did not have clinical evidence of active trachoma.

Five urethral isolates were serotyped. Of these, two were identified as type E and the other three as serotypes G, H, and K.

Discussion

The culture results were invalid in 27% of patients. This figure was appreciably higher than the rates of 4-8% from urethral specimens collected in London and tested in our laboratory.[9 10] This difference may have been due to variation in the bacterial flora of the urethra or sensitivity to streptomycin and vancomycin used in the transport medium.[4]

The chlamydial isolation rate of 9% obtained in Iranian patients was the same as that obtained from men with gonorrhoea attending an STD clinic in Kenya,[11] but it was much lower than the isolation rates of 30-50% commonly found in patients with NGU in Western countries.[3 9 10 12] This low isolation rate probably reflected the type of patients, the severity of the disease, or the treatment they received during their present infection.

The patients included in the study consecutively attended the clinic and gave a history of urethral symptoms. Routine laboratory investigations showed that in nearly half of the patients the urethritis was very mild or trival (<10 polymorphonuclear cells/high-power field at × 400 magnification in the urethral smears). Studies have shown that in patients with a mild urethritis the chlamydial isolation rate is much lower than in patients with moderate to severe disease.[9]

TABLE II *Titres of type-specific antichlamydial antibodies in sera from 147 men*

Type of antibody	*Total No (%) of positive sera*	*Antibody titres:* 8	16	32	64	128	256	*Total No (%) (IgG and/or IgM)*
D-K								
IgG	14 (10)	ND	8	3	1	1	1	23 (15·7)
IgM	17 (12)	12	3	2	0	0	0	
A-C								
IgG	7 (5)	ND	1	4	1	1	0	8 (5%)
IgM	1 (1)	1	0	0	0	0	0	

ND = not done

Of the patients studied, 133 (77%) had been treated within the previous year for suspected or confirmed urethritis with drugs active against chlamydia. This finding suggests that these patients may have had recurrences or reinfections during this period. Studies by Alani *et al*[9] have shown that in patients with a history of previous episodes of urethritis chlamydial isolation rates are much lower than in patients infected for the first time.

Of 48 patients who had had treatment within one month of the examination, chlamydia were isolated from only one (2%) whereas type-specific IgM against serotypes D to K, indicating current infection, was found in 14 (29%); this difference was statistically significant ($P<0{\cdot}001$, McNemar test). In studies in Western countries, however, chlamydia have been isolated from the majority of patients in whom specific IgM is found in the serum.[13, 14] These results suggest that in Iranian patients shedding of chlamydia from the urethra may have been suppressed by recent treatment with antichlamydial drugs.

C trachomatis serotypes E, G, H, and K were isolated from Iranian patients. In the Western world serotypes D, E, F, G, and K are common causes of genital infection whereas serotype H is less common.[13, 14].

Serological evidence of current infection with serotypes A to C (trachoma agent) was found in three patients who had no evidence of active trachoma. In this study we were not able to isolate serotypes A to C from the urethra and therefore could not prove that these serotypes caused urethritis. Serotypes B and C have, however, been isolated from the genital tract.[13, 14].

Examination of clinical records of the patients showed that 83% of them had had sexual intercourse with prostitutes within one month of the onset of urethritis. This finding suggests that in Teheran prostitutes are a major reservoir of chlamydial genital infection.

The results of this study indicate that in Iran chlamydial genital infection due to *C trachomatis* serotypes D to K does occur among the urban population. Although the chlamydial isolation rate in our patients was low we believe that had we selected patients with untreated primary but moderate to severe NGU the chlamydial isolation rate would have been much higher and probably nearer to the rates commonly found in Western countries.

We are grateful to the Dean of the School of Public Health, Teheran, and the medical directors of the Nejat STD clinic in Teheran for their support of this project. The study was supported by grants from an anonymous donor and the Dulverton Trust.

References

1. Pareek SS, Chowdhury MNH. Sexually transmitted diseases in Riyadh, Saudi Arabia. A study of patients attending a teaching hospital clinic. *Br J Vener Dis* 1981;57:443-5.
2. Darougar S, Treharne JD, Dwyer RStC, Kinnison JR, Jones BR. Isolation of TRIC agent (chlamydia) in irradiated McCoy cell culture from endemic trachoma in field studies in Iran. *Br J Ophthalmol* 1971;55:591-9.
3. Dunlop EMC, Vaughan-Jackson JD, Darougar S. Chlamydial infection, improved methods of collection of materials for culture from the urogenital tract and rectum. *Br J Vener Dis* 1972;48:421-4.
4. Gordon FB, Harper IA, Quan AL, Treharne JD, Dwyer RStC, Garland JA. Detection of chlamydia (Bedsonia) in certain infections of man. I Laboratory procedures: Comparison of yolk sac and cell culture for detection and isolation. *J Infect Dis* 1969;120:451-62.
5. Darougar S, Kinnison JR, Jones BR. Simplified irradiated McCoy cell culture for isolation of chlamydiae. In: Nichols RL, ed. *Trachoma and Related Disorders caused by Chlamydial Agents.* Amsterdam; Excerpta Medica, 1971: 64-70.
6. Treharne JD, Darougar S, Jones BR. Modification of the micro-immunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 1977;30:510-7.
7. Treharne JD, Katzenelson E, Davey SJ, Gray SJ. Comparison of serotyping subgroup A chlamydial isolates by a one-way cross-reaction and by a two-way cross-reaction immunofluorescence test. In: Nichols RL, ed. *Trachoma and Related Disorders caused by Chlamydial Agents.* Amsterdam: Excerpta Medica, 1971: 289-304.
8. Darougar S, Treharne JD, Minassian DC, El-Sheikh H, Dines RJ, Jones BR. Rapid serological test for diagnosis of chlamydial ocular infections. *Br J Ophthalmol* 1978;62:503-8.
9. Alani MD, Darougar S, Burns DCMacD, Thin RN, Dunn H. Isolation of *Chlamydia trachomatis* from the male urethra. *Br J Vener Dis* 1977;53:88-92.
10. Dunlop EMC, Vaughan-Jackson JD, Darougar S, Jones BR. Chlamydial infection; incidence in "non-specific" urethritis. *Br J Vener Dis* 1972;48:325-8.
11. Nsanze H, Waigwa SRN, Mirza N, Plummer F, Roelants P, Piot P. Chlamydial infection in selected populations in Kenya. In: Mårdh P-A, *et al* (eds.) *Chlamydial Infections,* Amsterdam: Elsevier Biomedical Press, 1982;421-4.
12. Holmes KK, Handsfield HH, Wang S-P, Wentworth BB, Turk M, Anderson JB, Alexander ER. Etiology of non-gonococcal urethritis. *N Engl J Med* 1975;292:1199-206.
13. Wang S-P, Grayston JT, Kuo CC, Alexander ER, Holmes KK. Serodiagnosis of *Chlamydia trachomatis* infection with the micro-immunofluorescence test. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC: American Society for Microbiology, 1977: 237-48.
14. Treharne JD, Dines RJ, Darougar S. Serological responses to chlamydial ocular and genital infections in the United Kingdom and Middle East. In: Hobson D, Holmes KK, eds. *Non-gonococcal Urethritis and Related Infections.* Washington DC:American Society for Microbiology, 1977:249-58.

Br J Vener Dis 1982;**58**:377-80

Pathogenic microbial flora of genital ulcers in Sheffield with particular reference to herpes simplex virus and Haemophilus ducreyi

G R KINGHORN, S HAFIZ, AND M G McENTEGART

From the Department of Genitourinary Medicine, Royal Infirmary; and the Department of Medical Microbiology, University of Sheffield Medical School, Sheffield

SUMMARY The pathogenic microbial flora of genital ulcers in 161 (80 men and 81 women) unselected patients was studied prospectively. In only one case was *Treponema pallidum* responsible whereas herpes simplex virus was considered to be the cause of 130 (80·8%) genital ulcers. *H ducreyi* was isolated from 46 (28·6%) patients, most commonly as a secondary pathogen in herpetic lesions. Two or more pathogens were isolated from the ulcers in 67 (41·6%) patients, and in 21 (13%) patients no pathogens were isolated.

Our results indicate an urgent need for antiviral treatment to reduce the local reservoir of genital herpes, challenge traditional concepts about the prevalence of *H ducreyi* in Britain, and call for a reappraisal of its role in the causation of genital ulcers.

Introduction

Recent studies[1 2] (unpublished data) of the microbial aetiology of genital ulceration show notable differences, particularly as regards the causes of non-syphilitic ulcers. This may partly reflect differences in technique but also genuine geographical differences in the incidences of genital pathogens. Whereas chancroid is considered to be endemic in tropical countries[3] and genital herpes is infrequently reported, the converse is true in developed Western countries. In Britain, where chancroid has until recently been rarely diagnosed, and even then usually as an imported infection, the incidence of genital herpes has risen to epidemic levels in the past decade. The advent of reliable cultural techniques[4 5] for the isolation of *Haemophilus ducreyi* in recent years has resulted in the reporting of localised outbreaks of chancroid in non-tropical countries[6-9]; recently we reported 22 cases in Sheffield[10] which were confirmed by culture using a modified haemin-containing medium.

In this study, we examined the pathogenic flora of genital ulcers in men and women presenting to the department of genitourinary medicine, Royal Infirmary, Sheffield, over a 12-month period.

Address for reprints: Dr G R Kinghorn, Department of Genitourinary Medicine, Royal Hallamshire Hospital, Sheffield S10 2JF

Accepted for publication 20 June 1980

Patients and methods

One hundred and sixty-one unselected patients (80 men and 81 women), who presented for the first time with discrete genital ulcers between 1 December 1980 and 30 November 1981, were routinely assessed. Exudate from the bases of ulcers was examined by darkfield microscopy for the presence of *Treponema pallidum* and collected with cotton-tipped swabs for both tissue culture inoculation to detect herpes simplex virus and bacteriological culture for *Haemophilus ducreyi,* other pathogenic aerobic and anaerobic bacteria, and *Candida* species. In addition, all patients had blood samples taken at their first visit for syphilis serology by the rapid plasma reagin (RPR) and the *Treponema pallidum* haemagglutination (TPHA) tests and for the detection of herpes simplex antibodies by complement-fixation, ELISA, or neutralisation techniques. In patients returning for follow-up serological tests for herpes antibody were repeated three weeks after the initial visit and for syphilis after three weeks, six weeks, and three months.

HERPES SIMPLEX VIRUS ISOLATIONS

Specimens of ulcer exudate obtained with cotton-tipped swabs were immediately inserted into viral transport medium and kept in the clinic refrigerator at 4°C before being transferred to the laboratory, normally within four hours. There they were stored at −70°C before being inoculated into monolayers of one or more of the following cell lines: African grey monkey (Vero), human epithelium (Hep 2), rabbit kidney (RKB), human embryo lung (Flow 2002), and hamster kidney (BHK 21). Inoculated monolayers were incubated at 37°C for up to 14 days and observed daily for the cytopathic effect of herpes.

In patients with negative culture results for herpes a fourfold rise in antibody titre in the convalescent sera was considered to be indicative of recent herpes simplex infection. Primary and recurrent episodes of herpes infection were distinguished by analysis of the patient's history, the severity and extent of the herpetic lesions, and the results of herpes antibody tests. Primary infections were diagnosed when there was no history of genital ulceration, there were numerous extensive skin lesions—usually bilateral and often accompanied by cervical or urethral lesions—and antibody titres were low or absent on initial serological testing. In contrast, patients with recurrent herpes almost always gave a history of episodes of genital ulceration, had few lesions usually confined to the skin, and had pre-existing antibodies at their first presentation usually at a high titre.

HAEMOPHILUS DUCREYI ISOLATIONS

Swabs containing ulcer exudate were inoculated directly at the time of examination on to two plates of modified haemin-containing medium, one of which contained 5% horse blood. The constituents of the base medium were as described[10] with the addition of gelatin (Oxoid) in a concentration of 4 g/l. Inoculated plates were transported to the laboratory, normally within two hours, where they were incubated in an atmosphere containing 5% carbon dioxide at 33°C for up to seven days.

H ducreyi isolates were initially recognised by their dark-brown colonies, which had a pronounced tendency to remain intact and could easily be distinguished from other organisms on the colourless medium. On the blood-containing medium the colonies produced α-haemolysis. The identity of the organisms was confirmed by Gram-staining, negative catalase, H_2S, indole, and carbohydrate fermentation test results, and positive test results for alkaline phosphatase and nitrate reduction. Our isolates conformed to the taxonomic requirements of Killian[11] and in all respects were identical to a reference strain (NTC 10945 type strain) and only differed from strains isolated from patients with classical chancroid in South Africa (kindly supplied by Dr P Piot, Institute Voor Tropische Geneeskunde, Antwerp, Belgium) and Kenya (kindly supplied by Dr A Ronald, Department of Medical Microbiology, Winnipeg, Manitoba, Canada), in being β-lactamase-negative when tested with chromogenic cephalosporin. All 16 isolates tested by intradermal injection of rabbits gave the pathogenic responses described by Hammond *et al.*[12]

AEROBIC AND ANAEROBIC BACTERIAL ANALYSIS

Swabs of ulcer exudate were normally transferred to the laboratory before being inoculated on to blood agar media (Oxoid blood agar base plus 10% lysed horse blood) and MacConkey's medium. When a delay greater than two hours between specimen collection and media inoculation was expected, swabs were placed in Amies transport medium. Inoculated plates were incubated for up to seven days both aerobically and anaerobically. Pathogenic isolates were identified using standard methods.[13]

Results

Separate analysis of the isolates from ulcers in men and women gave similar results (table). At least one pathogenic agent was found in 140 (87%) patients, and in 67 (41·6%) patients there were two or more pathogenic organisms in their ulcer flora.

TABLE *Pathogenic microbial flora isolated from the genital ulcers in 161 patients*

	Men (n=80)	*Women (n=81)*	*Total(%) (n=161)*
Herpes simplex virus	52	63	115 (71·4)
Treponema pallidum	1		1 (0·6)
Haemophilus ducreyi	24	22	46 (28·6)
Other aerobic bacteria	20	20	40 (24·8)
Staphylococcus pyogenes	6	6	12 (7·5)
β-haemolytic streptococci			
Group A	2	1	3 (1·9)
Group B	8	5	13 (8·1)
Group C	1	1	2 (1·2)
Group G	4	3	7 (4·3)
Enterococci	1	5	6 (3·7)
Coliforms	3	5	8 (5·0)
Neisseria gonorrhoeae		1	1 (0·6)
Anaerobic bacteria	5	4	9 (5·6)
Bacteroides species	3	3	6 (3·7)
Anaerobic cocci	3	2	5 (3·1)
Candida species	2	14	16 (9·9)
No pathogens isolated	13	8	21 (13·0)

HERPES SIMPLEX VIRUS

Herpes simplex virus was the most commonly isolated pathogen; in 52 patients it was the sole pathogen. In addition to the 115 patients in whom direct virus isolation from ulcers was successful, 15 other patients had a history of recurring genital ulceration with at least one episode of genital herpes confirmed by culture before their present presentation or serological evidence of current herpes simplex infection or both. In these culture-negative patients it is likely that the virus was the initiating cause of their genital ulceration. Thus, a total of 130 (80·8%) patients had genital herpes, of whom 78 had primary episodes and 52 recurrent episodes.

TREPONEMA PALLIDUM

T pallidum was the cause of genital ulceration in only one patient; he had acquired his infection in Mexico. No other patients had clinical, microscopical, or serological evidence of syphilis either at initial presentation or at subsequent follow-up.

HAEMOPHILUS DUCREYI

The commonest bacterial pathogen was *H ducreyi,* but in almost all of 46 patients infected with this organism there was evidence of a separate initiating cause for their genital ulcers; in 38 patients the organism was found in the lesions of genital herpes. Although *H ducreyi* was the sole pathogen isolated from ulcers in 10 patients, seven of these had a known history of recurrent genital herpes and the other three had secondary infection of ulcers caused by trauma, Behçet's syndrome, and excoriated penile psoriasis. The frequency of *H ducreyi* isolation was not appreciably different in patients with either herpetic or non-herpetic genital ulceration. The organism was isolated in 24 (30·8%) of 78 patients with primary herpes, 14 (26·9%) of 52 patients with recurrent herpes, and eight (25·8%) of 31 patients with non-herpetic ulceration.

H ducreyi was found in the ulcers of nine (47%) of 19 patients who had had sexual contacts in countries outside Britain in the four weeks before presentation compared with 37 (26%) of 142 who had had local contacts only; most *H ducreyi* infections had apparently been acquired locally. Moreover, in only two patients had there been contact with a known case of chancroid. Although ulcers containing *H ducreyi* were invariably tender with a purulent or necrotic base clinical features varied. Multiple ulcers (74%) and lymphadenitis (70%) were common in patients with *H ducreyi* infection but there was no case of bubo formation in this series.

OTHER AEROBIC BACTERIA

Other important aerobic bacteria were isolated from genital ulcers in 40 patients, but in only eight were they the sole pathogens isolated.

Only *Staphylococcus pyogenes* and β-haemolytic streptococci appeared to be primary pathogens. In the one patient in whom *Neisseria gonorrhoeae* was isolated from a vulval ulcer, herpes simplex virus and *H ducreyi* were isolated from the same genital lesion. It is, therefore, unlikely that she had true cutaneous gonorrhoea.

Anaerobic bacteria were never found in the absence of other microbiological causes of genital ulceration but may have had a secondary pathogenic role in some patients.

Candida species appeared to be secondary invaders of herpetic lesions in 11 patients but in five they may have intitiated genital ulceration by causing pruritus which lead to subsequent excoriation.

Discussion

In common with other recent studies we have found that genital ulcers often have a polymicrobial flora and frequently contain two or more pathogens. We found no obvious sex bias in the distribution of the major pathogens.

Herpes simplex virus was the most common isolate and probably the initiating cause of genital ulceration in 81% of our patients. This is a much higher frequency than in other studies and contrasts with 31-40% reported in North American studies,[1 14] and only 4% in Kenya.[2] Although the rapid increase in locally diagnosed cases of genital herpes during the past 10 years results partially from increased clinical awareness and improved methods of virus isolation, in the main the rise simply reflects the natural history of a condition where recurrences are common and effective treatment lacking. In Sheffield we have also found a high prevalence of HSV type 1 isolates[15] (possibly related to changes in sexual practice), contributing to the ever-increasing reservoirs of genital herpes in the community.

Infectious syphilis is rare within our relatively stable population because contact tracing and effective treatment of the few sporadic cases seen in recent years has rapidly removed the local reservoir of infection. It is interesting to compare the single case of imported treponemal ulceration in this study with the reported frequencies of 17% in Denver,[1] 12% in Seattle (unpublished data), and 11% in Kenya.[2]

H ducreyi was isolated from the genital ulcers of 28% of our patients, which compares with 1-2% in recent American studies[1] and 60% in Kenya.[2] In most infected patients the organism appeared to be a secondary invader rather than a primary pathogen. The sex distribution of infections was about equal and the great majority were locally acquired. As only two cases were linked, clearly our results were not simply due to a local outbreak of chancroid, although a local reservoir of individuals with asymptomatic genital carriage of *H ducreyi* may well exist. This view conflicts with the observations of others,[2 6 16] but evidence to support our contention was found in this study. In three men, all of whom presented initially with ulcers infected both with herpes simplex virus and *H ducreyi*, we were able to re-isolate *H ducreyi* from the preputial swabs after their ulcers had completely healed. All had been treated with saline washes alone. In a separate study, we have isolated *H ducreyi* from endocervical swabs taken from 11 women and the preputial swabs from three men, most of whom had no history of genital ulceration and none of whom had current genital ulcers.

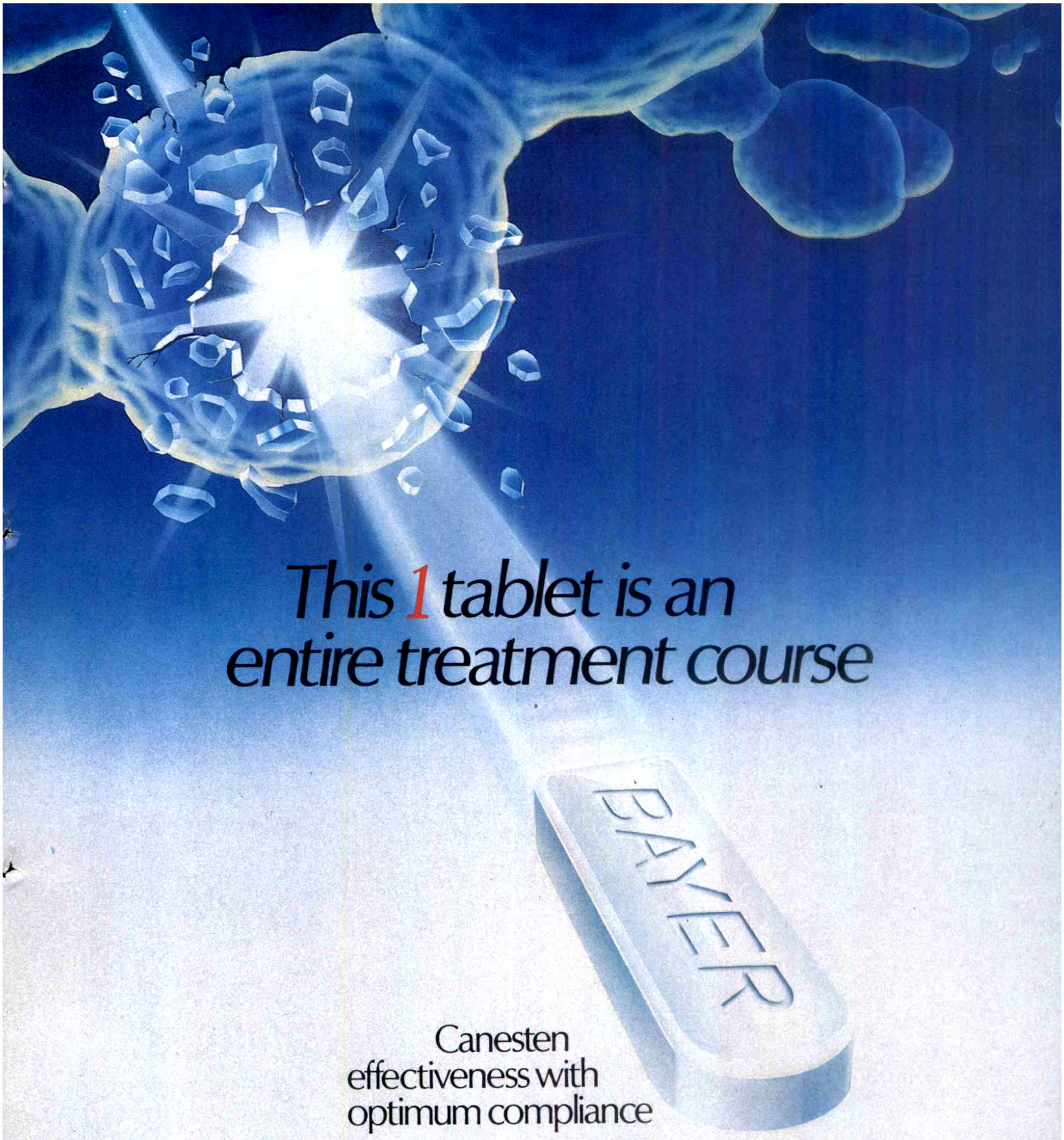
Organisms resembling *H ducreyi* have been seen on smears from chancroidal ulcers in previous studies,[1 17 18] and recently Ursi *et al*[19] described a "ducreyi-like bacillus", which they isolated from genital ulcers in 25 of 155 patients in Swaziland. This organism differed from *H ducreyi* in that it preferred a microaerophilic atmosphere, did not require X factor for growth, produced hydrogen sulphide and xylose fermentation, but gave a negative result to the alkaline phosphatase test. In another recent study Caine *et al* (unpublished data) suggested that *Haemophilus influenzae* and *Haemophilus parainfluenzae* may cause some genital ulcers. We did not routinely examine stained smears from ulcers, because of the low specificity and sensitivity of this investigation, and are satisfied that our isolates were indeed *H ducreyi*, which have a high requirement for haemin and share identical cultural and biochemical characteristics with test strains of the organism. Our local strains may possibly have had a lower pathogenicity in humans than those found in the tropics, although we have so far failed to show any diminished pathogenicity in rabbits. We are aware that strains isolated from typical cases of chancroid in Africa are frequently β-lactamase producers[2 20] whereas our local strains are not; we are, therefore, investigating them for other possible differences in plasmid content and antimicrobial sensitivity. Although our methods for isolating other aerobic and anaerobic bacteria were less sensitive than in other studies,[1 14] which detected commensal as well as pathogenic organisms, we nevertheless found bacteria other than *H ducreyi*, which were fairly common secondary invaders of ulcers caused by herpes simplex virus and trauma but were relatively rare as primary pathogens. In some cases antibiotic treatment was necessary to eradicate these organisms and so ensure healing of the ulcer.

This study shows that the most frequent initiating cause of genital ulceration in Sheffield is herpes simplex virus and that herpetic lesions are frequently subject to secondary infection with *H ducreyi* and other bacteria. The immediate and long-term morbidity associated with genital herpes makes accurate diagnosis essential, and the increasing local reservoir of genital herpes in this community necessitates the urgent development of effective antiviral treatment.

We are particularly grateful to the medical and nursing staff of the department of genitourinary medicine for their co-operation in collecting specimens; Dr I Barton, the staffs of the Sheffield Public Health Service, and the department of bacteriology, Royal Hallamshire Hospital, for microbiological assistance; and Elise Welsh and Hazel Bland for their secretarial help.

References

1. Chapel T, Brown WJ, Jeffries C, Stewart JA. The microbiological flora of penile ulcerations. *J Infect Dis* 1978; **137**: 50-6.
2. Nsanze H, Fast MV, D'Costa LJ, Tukei P, Curran J, Ronald AR. Genital ulcers in Kenya: clinical and laboratory study. *Br J Vener Dis* 1981; **57**: 378-81.
3. Gaisin A, Heaton CL. Chancroid: alias the soft chancre. *Int J Dermatol* 1975; **14**: 188-97.
4. Sottnek FO, Biddle JW, Kraus SJ, Weaver RE, Stewart JA. Isolation and identification of *Haemophilus ducreyi* in a clinical study. *J Clin Microbiol* 1980; **12**: 170-4.
5. Hammond GW, Lian CJ, Wilt JC, Ronald AR. Comparison of specimen collection and laboratory techniques for isolation of *Haemophilus ducreyi*. *J Clin Microbiol* 1978; **7**: 39-43.
6. Lykke-Olsen L, Larsen L, Pedersen TG, Gaarslev K. Epidemic of chancroid in Greenland 1977-78. *Lancet* 1979; **i**: 654-5.
7. Nayyar KC, Stolz E, Michel MF. Rising incidence of chancroid in Rotterdam: epidemiological, clinical, diagnostic and therapeutic aspects. *Br J Vener Dis* 1979; **55**: 439-41.
8. Hammond GW, Slutchuk M, Scatliff J, Sherman E, Wilt JC, Ronald AR. Epidemiologic, clinical, laboratory and therapeutic features of an urban outbreak of chancroid in North America. *Rev Infect Dis* 1980; **2**: 867-9.
9. Handsfield HH, Totten PA, Fennel CL, Falkow S, Holmes KK. Molecular epidemiology of *Haemophilus ducreyi* infections. *Ann Intern Med* 1981; **95**: 315-8.
10. Hafiz S, Kinghorn GR, McEntegart MG. Chancroid in Sheffield: a report of 22 cases diagnosed by isolation of *Haemophilus ducreyi* in a modified medium. *Br J Vener Dis* 1981; **57**: 382-6.
11. Killian M. A taxonomic study of the genus *Haemophilus* with the proposal of a new species. *J Gen Microbiol* 1976; **93**: 9-62.
12. Hammond GW, Lian CJ, Wilt JC, Ronald AR. Antimicrobial susceptibility of *Haemophilus ducreyi*. *Antimicrob Agents Chemother* 1978; **13**: 608-12.
13. *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd ed. Cambridge: Cambridge University Press. 1974.
14. Barile MF, Blumberg JM, Kraul CW, Yaguchi R. Penile lesions among US Armed forces personnel in Japan. *Arch Dermatol* 1962; **86**: 273-81.
15. Barton IG, Kinghorn GR, Najem S, Al-Omar LS, Potter CW. Incidence of HSV-I and HSV-2 isolated from a group of patients with herpes genitalis in Sheffield. *Br J Vener Dis* 1982; **58**: 44-7.
16. Ronald AR, Wilt JC, Albritton WL. *Haemophilus decreyi*. In: *Non-gonococcal Urethritis and Other Sexually Transmitted Diseases*. Geneva: WHO 1978.
17. Deacon WE, Albritton DC, Olansky S, Kaplan W. VDRL chancroid studies. I A simple procedure for the isolation and identification of *Haemophilus ducreyi*. *J Invest Dermatol* 1956; **26**: 399-406.
18. Leibovitz A. An oubreak of pyogenic penile ulcers associated with a microaerophilic streptococcus resembling *Haemophilus ducreyi*. *Am J Syph Gonorr Vener Dis* 1954; **38**: 303-15.
19. Ursi JP, van Dyck E, Ballard RC, Jacob W, Piot P, Meheus AZ. Characterisation of an unusual bacterium isolated from genital ulcers. *J Gen Microbiol* 1982; **15**: 97-103.
20. Maclean IW, Bowden GHW, Albritton HL. TEM type β-lactamase production in *Haemophilus ducreyi*. *Antimicrob Agents Chemother* 1980; **17**: 897-900.



This 1 tablet is an entire treatment course

Canesten
effectiveness with
optimum compliance

Canesten® 1

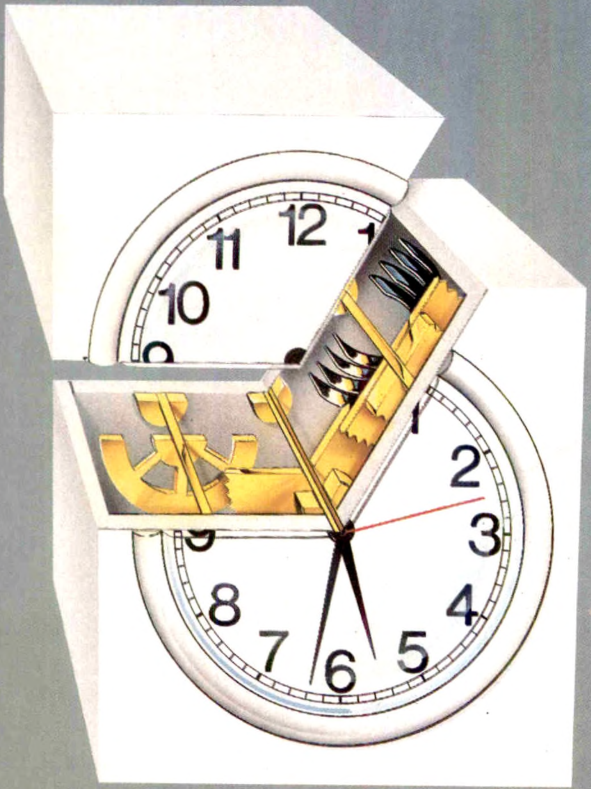
clotrimazole

Singularly effective in vaginal candidiasis

Prescribing Information: Presentations. *Vaginal Tablets* Canesten is available either as 200mg vaginal tablets in packs of three with applicator or as 100mg vaginal tablets in packs of six with applicator, or as a single 500mg vaginal tablet with applicator (Canesten 1). *Vaginal Cream* 2.0% clotrimazole cream in tubes of 35g with six applicators. *Canesten Duopak* Six 100mg vaginal tablets with applicator plus 20g cream containing 1.0% clotrimazole. **Dosage.** *Vaginal Tablets 100mg* Two vaginal tablets should be inserted at night for three consecutive days. Alternatively, one vaginal tablet daily for six days. *Vaginal Tablets 200mg* One vaginal tablet should be inserted at night for three consecutive days. *Vaginal Tablets 500mg (Canesten 1)* A single vaginal tablet to be inserted preferably at night. *Vaginal Cream* One filled applicator (5g) should be inserted twice daily for three consecutive days or once daily for six consecutive days preferably at night. *Duopak* Vaginal Tablets 100mg as above. The cream should be applied night and morning to the vulva and surrounding area and/or to the sexual partner's penis to prevent re-infection. **Side effects.** Rarely patients may experience transient local mild burning or irritation. **Basic NHS Costs.** 6 x 100mg vaginal tablets with applicator £2.13; 3 x 200mg vaginal tablets with applicator £1.99; 1 x 500mg vaginal tablet with applicator £2.50: Cream 35g with applicators £4.16; Duopak £3.47. **Product Licence Numbers.** *Vaginal Tablets 100mg,* PL0010/0015, *Vaginal Tablets 200mg,*

Further information is available from:
Bayer UK Limited
Pharmaceutical Division
Haywards Heath
West Sussex RH16 1TP
Telephone: Haywards
Heath (0444) 414161

*Registered trade mark of Bayer, Germany.



Syfhatect* in Syphilis detection

Screen and then titrate within four hours

Haemagglutination test using antigen-coated turkey cells, for the detection of antibodies to *Treponema pallidum*.

No pre-testing steps such as pre-absorption: results are readable in 2 hours.

Simple, rapidly performed five-step protocol.

Consistent, easy-to-read results.

Close correlation with FTA-Abs results warrants full confidence in use.

Compact and comprehensive kit includes antigen-coated and control red cells, positive and negative control sera, buffer, disposable microtitration plates and lids, droppers and bulbs, and direction leaflet.

Reconstituted cells stored at 2°–8°C have 5 days life and can be stored frozen for up to 28 days.

Ordering:
Please quote the code when ordering.

Code:
VD 31. 120 tests. Kit presentation. 18 mths life. Store at 2°–8°C.

*Trade mark

Wellcome Diagnostics
A Division of The Wellcome Foundation Limited Temple Hill Dartford England DA1 5AH

Please send me your fully comprehensive brochure on Syfhatect (which also details Syfacard-R*, the 10-minute screening test).

(Complete in capital letters)

Name

Address

Wellcome Diagnostics
A Division of The Wellcome Foundation Limited Temple Hill Dartford England DA1 5AH

V.D.1

Br J Vener Dis 1982;**58:**381-6

Preparation and immunogenicity of vaccine Ac NFU$_1$ (S$^-$) MRC towards the prevention of herpes genitalis

G R B SKINNER,* C B J WOODMAN,* C E HARTLEY,* A BUCHAN,* A FULLER,* J DURHAM,* M SYNNOTT,* J C CLAY,† J MELLING,‡ C WIBLIN,‡ AND J WILKINS‡

*From the *Department of Medical Microbiology, The Medical School, Birmingham; the †Special Clinic, General Hospital, Birmingham; and the ‡Centre for Applied Microbiological Research, Porton Down, Salisbury, Wiltshire*

SUMMARY A subunit antigenoid vaccine, Ac NFU$_1$ (S$^-$) MRC, was used to prevent primary herpes genitalis in 60 subjects considered to be at risk of this infection. There was no evidence of serious local or general side effects.

Neutralising antibody responses were detected in 59% and 90% of subjects receiving the low and high doses of vaccine respectively; immunoprecipitating antibody was detected at a lower frequency, namely in 23% and 43% of subjects receiving the low and high doses respectively. After a mean follow-up period of 18 months none of the vaccinated subjects contracted herpes genitalis after completing the vaccination course.

Introduction

Herpes genitalis is an acutely painful sexually transmitted disease (STD) whose prevalence is rapidly increasing throughout the United Kingdom[1]; moreover, as patients will remain infectious during and to some extent between clinical recurrences the prevalence of this disease, unlike other STDs, will, if left unchecked, increase. Similar anxieties arise with the complications of this infection; for example, the management of the pregnant patient with herpes genitalis is now a regular problem in our maternity centre. Finally, the large body of molecular and both retrospective and prospective epidemiological evidence associating this infection with the later development of preinvasive and invasive cervical carcinoma[2-8] emphasises the urgency of instituting measures for preventing this infection not only for the individual patient but for the community as a whole.

The feasibility of immunisation against herpes simplex infection was first shown in rabbits by Lipchutz[9] and more recently against primary type 2 herpes virus infection using subunit or inactivated type 1 or type 2 vaccine preparations.[10-15] Of particular importance are a number of studies reporting varying degrees of protection against latent type 2 ganglionic infection in mice immunised with live or inactivated type 2 virus vaccines[16-18] or type 1 vaccine preparations.[19-21]

It is curious therefore how little effort has been directed towards the prevention of primary herpes simplex infections by vaccination of as yet uninfected subjects. The relative ease of autoreinoculation of human subjects with live herpes virus[22-24] has been discouraging as was an unsuccessful attempt to immunise 7-11-month-old orphaned children against primary type 1 herpes infections.[25] There must, however, be reservations about the immunogenic potency of the vaccine preparations in this study and the criteria of assessment of vaccine efficacy.

The present study reports the preparation, immunological response, and clinical outcome after immunisation with vaccine Ac NFU$_1$ (S$^-$) MRC of 34 seronegative and 26 seropositive subjects at risk of genital herpes virus infection.

Subjects and methods

The regular sexual consorts of 60 patients with recurrent genital herpes (index cases) were offered vaccination. The frequency, duration, severity, and

Address for reprints: Dr G R B Skinner, Department of Medical Microbiology, The Medical School, Birmingham B15 2TJ

Accepted for publication 3 June 1982

location of herpetic disease in the index cases, the frequency of sexual relations, mode of contraception, and duration of the consortship were recorded. Parallel information was obtained from a control group of consorts at risk, namely the regular sexual partners of 20 consecutive patients who attended our clinic with primary or recurrent herpes genitalis before our preventative vaccination programme was begun. There was no history of herpes genitalis in either the vaccinated or unvaccinated subjects.

The vaccinated group of consorts (29 men and 31 women) had a mean age of 23·1 years; the unvaccinated subjects (12 men and eight women) had a mean age of 24·2 years. The difference in the socioeconomic status of the vaccinated and unvaccinated consorts was 16%, 57%, and 27% in the high (I and II), middle (III), and low (IV and V) socioeconomic groups respectively compared with 45%, 40%, and 15%. The vaccinated subjects tended therefore toward a lower socioeconomic status.

The sociosexual inter-relationships between the vaccinated and unvaccinated groups seemed comparable although in a study of this size it was not feasible to compare precisely certain sex-related variables—for example, frequency, duration, and mode of sexual relations in a given index-consort coupling. In both vaccinated and unvaccinated groups about 30% of consorts were the marital partners and the remaining 70% were the regular sexual partners of the index case; similarly the risk of exposure to herpes infection from the index case was comparable where the mean frequency of recurrences was 6·6 per year in the vaccinated and 6·0 per year in the unvaccinated consort group. There were no detectable differences in contraceptive practice or precautionary measures during herpetic recurrences between the vaccinated and unvaccinated subjects.

The subjects were followed-up for a mean of 18 months (range 4-26 months) with monthly assessment during the first three months, a further assessment at six months, and assessment at six-monthly intervals thereafter. A history of clinical features of herpes genitalis was sought at each visit; subjects were also asked to record any signs or symptoms which might be related to herpes genitalis and to report them to the responsible medical practitioner. As the principal aim of this study was to investigate the prevention of clinically overt herpes genitalis, vaginal material for virological examination was not taken unless there was clinical suspicion of herpes genitalis.

VACCINATION PROCEDURE

Two subcutaneous vaccinations were given at monthly intervals in the deltoid muscle. For the first 50 vaccinations, a test dose of 0·05 ml was administered; as no untoward reactions were observed, this precaution was omitted unless there were particular indications—for example, a history of allergic reactions, anaphylaxis, or penicillin hypersensitivity.

Two doses of vaccine were used. Thirty-nine consorts were immunised with the lower dose of 2×10^7 cell equivalents; as experience with the vaccine increased 21 consorts, identified as seronegative for neutralising antibody to type 1 or type 2 herpes simplex virus, were immunised with the higher dose of 10^8 cell equivalents.

Serum samples were obtained before vaccination and one month after the last vaccination.

Cells

MRC 5 cells, a human embryonic lung cell line, obtained from the National Institute for Biological Standards and Controls, London, were used for vaccine production and for the isolation, cloning, and preparation of virus stocks for vaccine production.

BHK 21, a stable line of baby hamster kidney cells,[26] were used for virus titration.

Virus strains

Type 1 strain (troisbell) was used for vaccine preparation. The strain was isolated and cloned three times in MRC 5 cells. Virus stocks were stored in serum-free medium. Type identification of virus strains was confirmed by neutralisation kinetics and Ouchterlony gel diffusion tests.[27]

Strains HFEM and strains 3345 were used respectively as prototype type 1 and type 2 strains for virus antigen preparation and for neutralisation tests.

Virus antigen

Type 1 and type 2 herpes simplex virus antigens for Ouchterlony immunodiffusion tests were prepared by high multiplicity infection of BHK 21 cells.[28]

VACCINE PREPARATION

Vaccine was prepared as previously described.[28a] MRC 5 human embryo lung cells were infected with strain troisbell of herpes simplex virus. Cell nuclei were removed by low-speed centrifugation after treatment of the cells with Nonidet NP40, which also strips important virus antigens from the envelope of the virus particles; formaldehyde was added to inactivate viral and host cell DNA and to stabilise virus antigens during subsequent preparative procedures. Virus particles, which although inactivated might contain biologically active DNA, were then removed by ultracentrifugation over 20% sucrose at 85 000 × *g* for five hours. The protein constituents of the vaccine preparation were precipitated by cold acetone and the precipitate washed in acetone, which was then removed by evaporation. The dried pellet was stored at −70°C in a dehydrated container or for short-term storage at 4°C.

The safety, immunogenicity, and protective efficacy of vaccine preparations has been previously reported.[11-13, 29, 30] Before inoculation into human subjects vaccine batches were tested by subcutaneous inoculation of 0·05 ml (5×10^6 cell equivalents) into the dorsal skin of newborn mice.

NEUTRALISATION TESTS

Sera were tested by neutralisation kinetics at a final 1/10 dilution in PBS. K values were calculated for each serum as previously described.[4]

IMMUNODIFFUSION TESTS

Vaccine, antigen preparations, and human sera were tested in Ouchterlony immunodiffusion gels as previously described.[28]

POLYACRYLAMIDE GEL ELECTROPHORESIS

Vaccine preparations were analysed on 18 × 11 cm SDS-polyacrylamide slab gels cross-linked with NN' diallyl tartar-diamide. Procedures for fixing, staining, autoradiography, and calibration with molecular weight standards have been described.[31]

DETECTION OF POLYPEPTIDES BY REACTION WITH ANTISERUM AND IODINATED PROTEIN A

After thorough washing gels were incubated in appropriate dilutions of antiserum. Unreacted serum was removed by extensive washing and bound immunoglobulin detected by incubation with iodinated protein A. Radioactive proteins were detected by autoradiography.

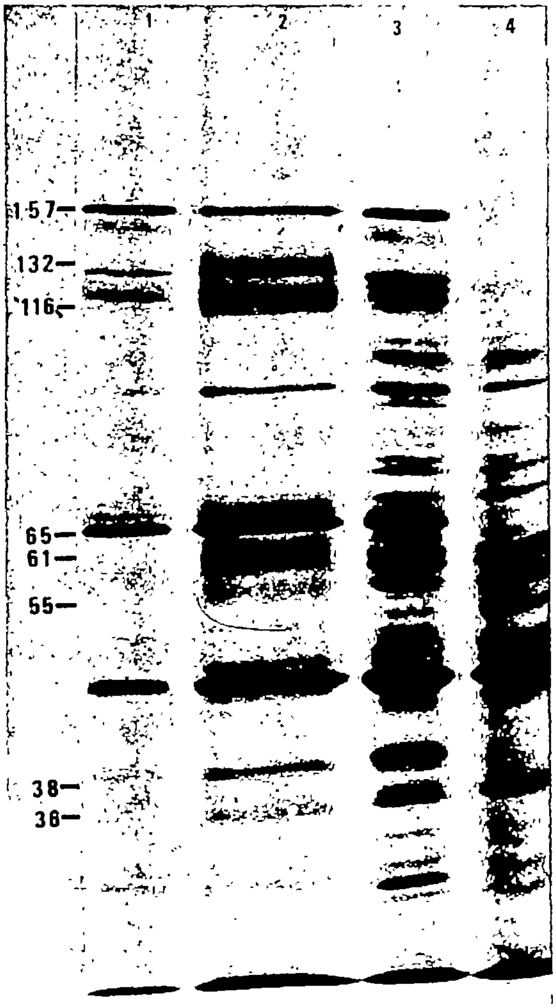


FIGURE *Polypeptide composition of vaccine at three stages of preparation by reaction with antiserum and iodinated protein A. Track 1, initial virus-infected MRC 5 cell extract after treatment with Nonidet NP40 and formaldehyde; track 2, final vaccine; track 3, virus-infected baby hamster kidney cells; and track 4, uninfected baby hamster kidney cells. (The numbers indicate apparent molecular weights × 10^{-3}.)*

Results

EFFICACY OF VACCINE

Polypeptide composition

The polypeptide composition at the three stages of preparation procedure is shown in the figure. The vaccine polypeptide profile is virtually indistinguishable from the profile of type 1 virus infected cell extract, with easy identification of the polypeptides whose molecular weights correspond to the polypeptides of the virus particle[31]; in particular, glycosylated polypeptides in the molecular weight range 118 000-132 000 and 55 000-63 000 were identified in every vaccine preparation. These are components of the virus envelope and have been shown to stimulate both type-specific and type-common neutralising antibodies in experimental rabbits.[32, 33] The antigenicity of virus polypeptides in the vaccine has been confirmed by in-situ identification on polyacrylamide gels using hyperimmune rabbit antiserum to type 1 herpes virus infected cells.

Immunoprecipitating virus antigens

Vaccine preparations were routinely tested against hyperimmune type 1 antisera in Ouchterlony immunodiffusion. Under these circumstances one or two immunoprecipitins were detected; one of these immunoprecipitins was invariably band II antigen (glycoprotein d), an antigen of major significance in virus neutralisation.[32]

STUDIES IN HUMAN SUBJECTS

Neutralising antibody

In consorts receiving the low dose of vaccine there was a significant neutralising antibody response in 10 of 13 seronegative subjects and in 13 of 26 seropositive subjects; after the high dose 19 of 21 subjects developed neutralising antibody with a mean neutralisation rate constant of 0·37 against type 1 and 0·29 against type 2 virus (table).

Immunoprecipitating antibody

One immunoprecipitin line was identified in three of 13 and in nine of 21 sera from consorts receiving the low and high doses respectively. In five of these sera, two immunoprecipitin lines were detected. One of the immunoprecipitin lines was common to all sera that reacted and was identified as anti-band II. None of the sera reacted against uninfected baby hamster kidney cell extract.

Clinical efficacy

At the time of writing, 60 vaccinated patients have been followed up for 920 patient months with a mean length of consortship of 18 months (range of 4-26 months). After completing the vaccination course, none of the consorts has developed clinical evidence of herpes genitalis; it is also noteworthy that five female subjects have reported unprotected sexual intercourse during recurrence of vulvo-vaginal herpetic disease with five male consorts, three of whom had sero-converted after low-dose vaccination and two of whom had not developed neutralising antibody after low-dose vaccination; to date there has been no clinical or serological evidence of herpes genitalis in any of these male consorts at risk. The reciprocal situation—that is unprotected exposure of vaccinated female subjects to herpes genitalis—has not as yet been reported in our study.

One consort, who had received her first vaccination one week previously, reported an episode which resembled a mild attack of herpes genitalis; there was a history of recent unprotected exposure to penile herpes from her marital partner. The patient did not attend for a further two weeks, at which time there was no clinical evidence of herpes genitalis although herpes simplex virus was isolated from the genital tract. While the features of this episode are not typical of primary herpes genitalis, and it is of course possible that herpes simplex virus was isolated after postcoital deposition and extracellular survival of virus in the genital tract, we feel obliged to record the episode as primary herpes genitalis occurring one week after first vaccination.

In 20 unvaccinated consorts, eight contracted primary herpes genitalis within one year of consortship, although three of the consorts contracted their infection from the same index case. Unfortunately, serological data were not obtained routinely from this unvaccinated group of consorts.

Side effects of vaccination

To date, in approximately 900 vaccinations including patients (not computed in this study) who received vaccination to modify recurrent herpetic disease[28a, 35] there have been no significant local or general side effects. Immediately after vaccination, most patients complained of a local "stinging sensation" lasting for 30-60 seconds and about 75% of patients had an erythematous reaction with swelling at the vaccination site for 24-72 hours; six vaccinations were followed by a transient "flu-like" syndrome which was at least temporally related to vaccination. Two seronegative and three seropositive consorts were vaccinated in early pregnancy without ill effect to mother or infant.

Discussion

A group of 60 consorts at risk were immunised with antigenoid vaccine Ac NFU$_1$ (S$^-$) MRC to prevent

TABLE *Details of vaccination response in 60 sexual consorts of patients with recurrent herpes genitalis*

Vaccine	No in group	No with neutralising antibody response*	Mean k value				No with Immunoprecipitating antibody
			Before vaccination		After vaccination		
			HSV type 1	HSV type 2	HSV type 1	HSV type 2	
High-dose							
Seronegative	21	19	0	0	0·37	0·29	9
Low-dose							
Seronegative	13	10	0	0	0·18	0·13	3
Seropositive	26	13	0·49	0·20	0·69	0·39	ND

*Neutralising antibody responses were recorded as positive when there was a significant increase in k value against both type 1 and type 2 herpes virus

ND = not detected

their contracting herpes genitalis. The vaccine contained polypeptides and glycoproteins whose antigenicity had clearly survived the preparative procedure as judged by analyses on polyacrylamide gels and Ouchterlony gel diffusion.

Neutralising antibody responses were obtained in a high proportion of subjects receiving the high dose of vaccine and in about one half of subjects receiving the lower dose. The detection of immunoprecipitating antibody albeit in a lower proportion of post-vaccination sera—which accords with our general experience of the relative prevalence of neutralising and immunoprecipitating antibody in the sera of the general population[28 36]—was encouraging and may provide a simple and convenient initial screening test for seroconversion in large-scale trials of vaccine efficacy.

The rationale of vaccination in seropositive consorts is an open question; however, as we have previously observed that vaccinated mice were significantly better protected than mice who had survived in a live infection[12] and, as in this study, there was a significant neutralising antibody response after vaccination in 13 of 26 subjects with pre-existing antibody (table), it does seem that vaccination offers a quantitatively or qualitatively different immunogenic stimulus. It is possible, for example, that treatment with Nonidet, formaldehyde, or acetone might impart adjuvant-like properties to vaccine polypeptides in terms of their in-vivo stability or immunogenic presentation.

The preventative efficacy of our vaccination programme has been evaluated by comparison with the rate of consort transmission computed prospectively from our experience during prevaccination years. This is not ideal but in some measure confounds the preventative influence of our advice and counselling towards minimisation of the risk of virus transmission between sexual consorts. Vaccinated and unvaccinated groups seemed generally comparable, except that the former were distributed towards a lower socioeconomic status which, if anything, might favour a higher rate of index to consort transmission. Some may have developed a subclinical virus infection but if this did occur, the absence of any clinical recurrences within 18 months is surprising and at worst a welcome modification of the usual clinical outcome.

While the duration of protection is traditionally less with inactivated vaccine preparations, it was encouraging that after immunisation with inactivated vaccines significant levels of protection were obtained for at least 18 months in mice and six months in rabbits and Rhesus monkeys.[35 37] In humans, as there are no unequivocal humoral or cell-mediated immunological correlates of protection against type 2 herpes virus infection, the long-term efficacy of vaccination must await the test of time. We intend to examine the temporal profile of various criteria of the humoral, local secretory, and cell-mediated immune response to varying vaccine dosages and immunisation schedules in the presence and absence of adjuvant and correlate this information with protective efficacy.

References

1. Anonymous. Epidemiology report. Sexually transmitted disease surveillance. *Br Med J* 1979;ii:1375-6.
2. Naib ZM, Nahmias AJ, Josey WE. Cytology and histopathology of cervical herpes simplex infection. *Cancer* 1966; **19**:1026-31.
3. Adam E, Kaufman RH, Melnick JL, Levy AH, Rawls WE. Seroepidemiological studies of herpes virus type 2 and carcinoma of the cervix IV. *Am J Epidemiol* 1974;**98**:77-87.
4. Skinner GRB. Transformation of primary hamster embryo fibroblasts by type 2 herpes simplex virus: evidence for a "hit and run" mechanism. *Br J Exper Pathol* 1976;**57**:361-76.
5. Skinner GRB, Whitney JE, Hartley C. Prevalence of type-specific antibody against type 1 and type 2 herpes simplex virus in women with abnormal cervical cytology; evidence towards pre-pubertal vaccination of sero-negative female subjects. *Arch Virol* 1977;**54**:211-21.
6. Eglin RP. MacLean AB, Sharp F, MacNab JCM, Clements JB, Wilkie NM. The detection of herpesvirus coded material in plastic cervical tissue. *Proceedings of the International Conference on Human Herpesviruses, Atlanta, Georgia, 1980.* New York: Elsevier, 1980.
7. Adelusi B, Naib Z, Muther J, Nahmias AJ. Epidemiologic studies relating genital herpes simplex virus (HSV) infection with cervical neoplasia—an update. *Proceedings of the International Conference on Human Herpesviruses, Atlanta, Georgia, 1980.* New York: Elsevier, 1980.
8. Coleman DV, Morse A, Beckwith P, Anderson MC. Prognostic significance of HSV antibody status in women with dysplasia of uterine cervix (CIN 1 or 2). *Br J Obstet Gynaecol* 1982, in press.
9. Lipchutz B. Untersuchungen uber die etiologic der Krankheiten der Herpesguruppe. *Arch Dermatol Syph* 1921; **136**:428-31.
10. Benda R, Bdaly V. Immunogenic properties of formolin herpes antigen prepared from cell cultures. *J Hyg Epidemiol Microbiol* 1973;**17**:237-49.
11. Skinner GRB, Williams DR, Buchan A, Whitney J, Harding M, Bodfish K. Preparation and efficacy of an inactivated subunit vaccine (NFU$_1$BHK) against type 2 herpes simplex virus infection. *Med Microbiol Immunol* 1978;**166**:119-32.
12. Skinner GRB, Williams DR, Moles AW, Sargent A. Prepubertal vaccination of mice against experimental infection of the genital tract with type 2 herpes simplex virus. *Arch Virol* 1980;**169**:39-51.
13. Skinner GRB, Buchan A, Hartley CE, Turner SP, Williams DR. The preparation, efficacy and safety of "antigenoid" vaccine NFU (S$^-$L$^+$) MRC toward prevention of herpes simplex virus infections in human subjects. *Med Microbiol Immunol* 1980;**169**:39-51.
14. Cappel R, de Cuyper F, de Brackeleer J. Benefit versus risk factors. International Symposium on Immunization. *Develop Biol Standard* 1979;**43**:381-6.
15. Kutinova L, Slichtova V, Rajcamy J, Vonka V. Immunogenicity of experimental subviral herpes simplex virus vaccine. *Proceedings of the International Conference on Human Herpesviruses, Atlanta, Georgia, 1980.* New York: Elsevier, 1980.
16. Walz MA, Price RW, Hayashi K, Katz BJ, Notkins AL. Effect of immunization on acute and latent infection of vaginal-uterine tissue with herpes simplex virus types 1 and 2. *J Infect Dis* 1977;**135**:744-52.

17. Salerno RD, Lehman ED, Conard PA, McGuire WR, Davies ME, Field AK. Development of nucleic acid free herpes glycoprotein subunit vaccines. *Proceedings of the International Conference on Human Herpesviruses, Atlanta, Georgia, 1980.* New York: Elsevier, 1980.

18. Hilleman RM, Larson VM, Lehman ED, Salerno RA, Conard PG, McLean AA. Subunit herpes simplex viruses vaccine. In: Nahmias AJ, Dowdle WR, Shinazi RF, eds. *The Human Herpesviruses.* New-York: Elsevier, 1981:503-6.

19. Asher LVS, Walz MA, Notkins AB. Effect of immunization on the development of latent ganglionic infection in mice challenged intravaginally with herpes simplex virus types 1 and 2. *Am J Obstet Gynecol* 1978;**131**:788-91.

20. Sturn B, Schneweis, K. Protective effect of an oral infection with herpes simplex virus type 1 against subsequent genital infection with herpes simplex virus type 2. *Med Microbiol Immunol* 1978;**165**:119-27.

21. McKendall RR. Efficacy of herpes simplex virus type 1 immunization in protecting against acute and latent infection by herpes simplex virus type 2 in mice. *Infect Immunol* 1977;**16**:717-19.

22. Lazar MP. Vaccination for recurrent herpes simplex infection. *Arch Dermatol* 1955;**73**:70-7.

23. Goldman L. Reaction of auto-inoculation for recurrent herpes simplex. *Arch Dermatol* 1961;**84**:1025-6.

24. Blank H, Haines GH. Experimental human reinfection with herpes simplex virus. *J Invest Dermatol* 1973;**61**:223-5.

25. Anderson S, Hamilton J, Williams S. Attempt to vaccinate against herpes. *Aust J Exp Biol Med Sci* 1950;**28**:579-84.

26. MacPherson I, Stoker M. Polyoma transformation of hamster cell clones—an investigation of genetic factors affecting cell competence. *Virology* 1962;**16**:147-51.

27. Thouless ME, Skinner GRB. Differences in the properties of thymidine kinase produced in cells infected with type 1 and type 2 herpes virus. *J Gen Virol* 1971;**12**:195-7.

28. Skinner GRB, Taylor J, Edwards J. Precipitating antibodies to herpes simplex virus in human sera; prevalence of antibody to common antigen (band II). *Intervirology* 1974;**4**:320-4.

28a. Skinner GRB, Woodman C, Hartley CE, *et al.* Early experience with antigenoid vaccine Ac NFU, (5) MRC towards prevention or modification of herpes genitalis. *Development of Biological Standardisation.* Basel, Switzerland: S Karger, 1982.

29. Skinner GRB. Pre-pubertal vaccination against herpes simplex virus infection towards prevention of cervical carcinoma. Blair Bell Memorial Lecture, Royal College of Obstetricians and Gynaecologists, London, 1980.

30. Skinner GRB. Herpes simplex infection: the trivial and the terrible. Ninian Faulkner Memorial Lecture, Dublin, 1981.

31. Heine JW, Honess RW, Cassai E, Roizman B. Proteins specified by herpes simplex virus. XII The virion polypeptides of type 1 strains. *Virol* 1974;**14**:640-51.

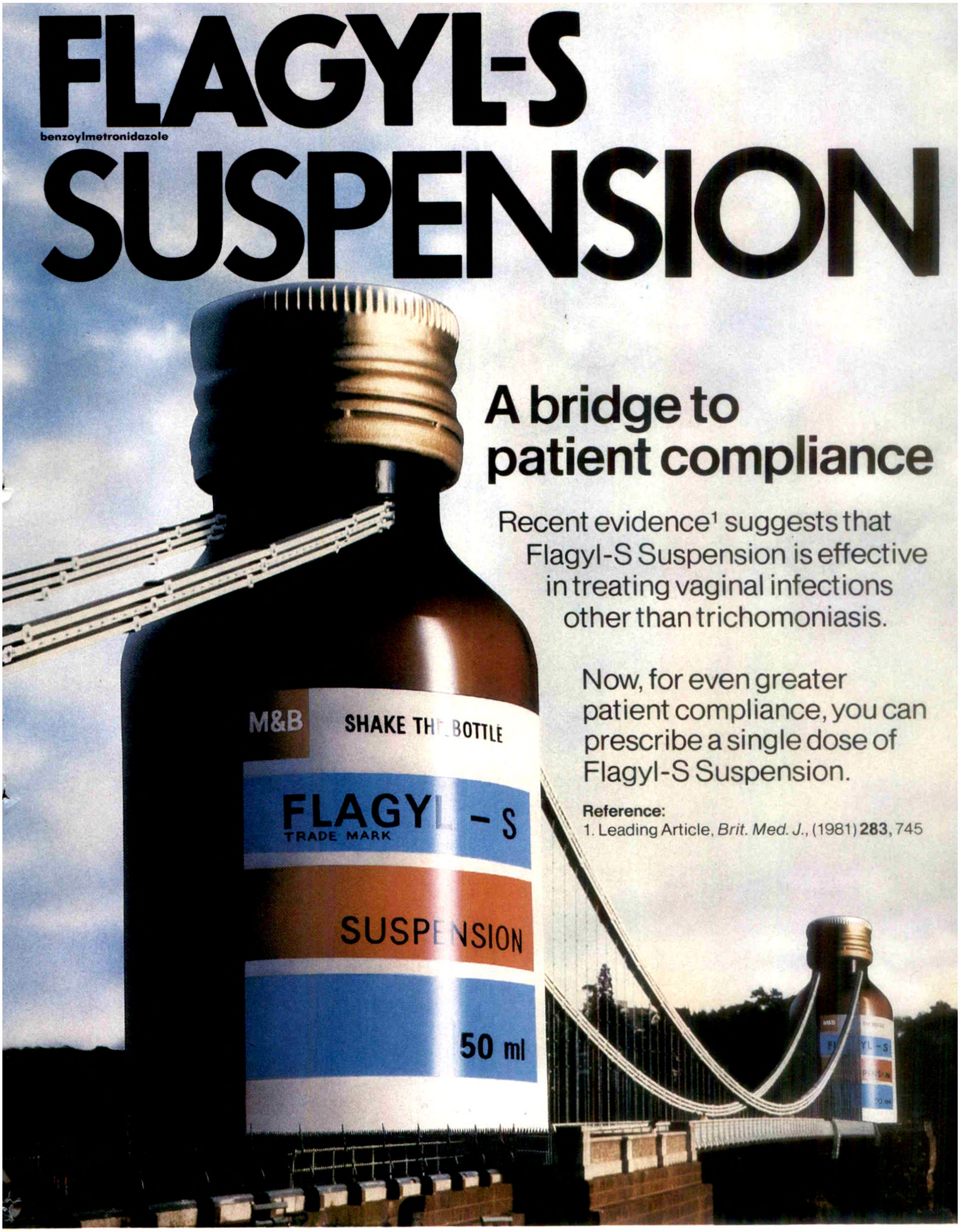
32. Sim C, Watson DH. The role of type specific and cross-reacting structural antigens in the neutralisation of herpes simplex virus types 1 and 2. *J Gen Virol* 1973;**19**:217-33.

33. Powell KL, Buchan A, Sim C, Watson DH. Type-specific proteins in herpes simplex virus envelope react with neutralising antibody. *Nature* 1974;**249**:360-61.

35. Skinner GRB, Woodman K, Hartley CE, Buchan A. The prevention of herpes simplex virus induced cervical carcinoma. *Proceedings of the Ninth College Study Group on Preclinical Neoplasia of the Cervix.* Fourth World Congress for Cervical Pathology and Colposcopy. London: Royal College of Obstetricians and Gynaecologists, 1982.

36. Babalola OG. An investigation of anti-herpes simplex virus antibodies in human sera by immunodiffusion, neutralisation and immunoprecipitation. *MSc Thesis,* University of Birmingham, 1981.

37. Carter CA, Hartley CE, Skinner GRB, Turner SP, Easty DL. Experimental ulcerative herpetic keratitis. IV Preliminary observations on the efficacy of a herpes simplex subunit vaccine. *Br J Ophthalmol* 1981;**65**:679-82.



FLAGYL-S

benzoylmetronidazole

SUSPENSION

A bridge to patient compliance

Recent evidence[1] suggests that Flagyl-S Suspension is effective in treating vaginal infections other than trichomoniasis.

Now, for even greater patient compliance, you can prescribe a single dose of Flagyl-S Suspension.

Reference:
1. Leading Article, *Brit. Med. J.*, (1981) **283**, 745

Prescribing Information: Presentation: Suspension: each 5ml containing 320mg benzoylmetronidazole equivalent to 200mg metronidazole. **Indications:** For the treatment of non-specific vaginitis and urogenital trichomoniasis. *Adult dosage:* Non-specific vaginitis: 50ml as a single dose or 10ml three times a day for seven days. Urogenital trichomoniasis: 2gm (50ml) as a single stat dose or 200mg three times a day for seven days. **Contra-indications:** There are no absolute contra-indications to the use of Flagyl. **Precautions:** Avoid alcohol or use in pregnancy, lactation; may enhance the effect of oral anticoagulants. The prescriber should also bear in mind the possibility that an accompanying gonococcal infection might persist in a symptomless state after trichomonas vaginalis has been eliminated. **Side-effects:** Occasional unpleasant taste, furred tongue and gastro-intestinal disturbances. Drowsiness, dizziness, headache, ataxia, skin rashes, pruritus, inco-ordination of movement, darkening of urine (due to a metabolite) has been reported but very rarely. A few instances of peripheral neuropathy have been reported during intensive and/or long–term treatment. **Presentation/Cost:** Suspension: Bottle of 50ml £2.05. Bottle of 125ml £4.80 (March 1982) PL 0012/0131
Further information available on request.

Flagyl-S is a trade mark of May & Baker Ltd.

M&B May&Baker May & Baker Ltd., Dagenham, Essex RM10 7XS.

Sexually Transmitted Diseases

Journal of the American Venereal Disease Association

Sexually Transmitted Diseases is a quarterly journal which contains original articles on all of the human sexually transmitted diseases. Also included are case reports, editorials, reviews, and historical articles. Clinical, epidemiological, and laboratory topics are covered as are basic research and applied research.

Individuals are invited to join the American Venereal Disease Association. Annual Dues are $30.00 outside the United States ($25.00 in the United States). Members receive *Sexually Transmitted Diseases* at no additional cost, and benefit from the other activities of the association.

Non-member and institutional subscriptions to *Sexually Transmitted Diseases* are available for $33.00 and $36.00 in the United States and $37.00 and $40.00 in all other countries.

William O. Harrison, M.D.
American Venereal Disease Association
P.O. Box 22349
San Diego, California 92122 USA

☐ Please enroll me as a Member of the American Venereal Disease Association. Enclosed is a U.S. Bank Draft or International Money order in the amount of $30.00 (U.S.) for my membership which includes a subscription to the Association's Official Journal *Sexually Transmitted Diseases*.

☐ I wish to enter a Non-Member or institutional subscription ($33.00 and $36.00 U.S., all other countries $37.00 and $40.00).
(Mail non-member or institutional subscriptions to J. B. Lippincott Company, East Washington Square, Philadelphia, Pa. 19105).

Name ____________________

____

Address ____________________

City ____________ State Country ____________ Zip ____________

Br J Vener Dis 1982; **58**: 387-93

Clinic diagnosis of anaerobic vaginosis (non-specific vaginitis)

A practical guide

A BLACKWELL AND D BARLOW

From the Department of Genitourinary Medicine, St Thomas's Hospital, London

SUMMARY Anaerobic vaginosis (non-specific vaginitis) can be readily diagnosed at the time of first attendance without recourse to expensive and time-consuming laboratory investigations. Diagnosis is based on careful history-taking, clinical examination, and simple investigations in the clinic. Although the presence of a malodorous vaginal discharge and the finding of clue cells on microscopy (in the absence of *Trichomonas vaginalis*) are sufficient for the diagnosis, a positive amine test and a vaginal pH of more than 5·0 are useful confirmatory tests.

Introduction

In spite of a wealth of recent reports on non-specific vaginitis there is a lack of practical advice to those working in genitourinary medicine and related fields as to how this diagnosis may be made at the time of the first examination. The guidelines suggested in this report resulted from a study started at St Thomas's Hospital, London, in 1979 into the clinical and microbiological aspects of non-specific vaginitis. Details of the bacteriological and other findings are published elsewhere.[1-4]

The population in this study (which continues) includes patients attending the department of genitourinary medicine and a local family planning clinic. The number of patients investigated has been necessarily limited by the constraints imposed by full anaerobic culture and identification of organisms in each case and to date includes 130 women.

Non-specific vaginitis is ill-named for three reasons. Firstly, it may be confused with non-specific genital infection; secondly it is associated with the presence of defined (or definable) bacteria; and, thirdly, it is not characterised by inflammation of the vagina. There have been waves of enthusiasm for the role of *Gardnerella* (née *Haemophilus*) *vaginalis* as the causative organism, and some agile arguments have been put forward to explain away its poor in-vitro sensitivity to metronidazole in the face of this antibiotic's manifest clinical effect.

In 1980 Spiegel *et al*[5] carried out a detailed analysis of the microbial flora associated with non-specific vaginitis. They considered that anaerobes might well act synergistically with *G vaginalis* to give rise to the condition, and many are now suspicious that the anaerobic component is responsible for some, if not most, of the clinical symptoms and signs. For the above reasons we prefer the term "anaerobic vaginosis" as a more apt and descriptive name for the disease.

Our practical guidelines beg the question of the relative roles of *G vaginalis* and anaerobic bacteria in the pathogenesis of this condition and are independent of the need for expensive and time-consuming laboratory investigations. The equipment needed is already available in most departments of genitourinary medicine and comprises a bright-field microscope with ×40 and ×100 objectives, facilities for Gram-staining, pH paper (Whatman narrow range pH 4-6), 5-10% potassium hydroxide, and wooden orange sticks for mixing.

Anaerobic vaginosis

The symptoms and findings of anaerobic vaginosis include:

(1) Genital malodour, often described as 'fishy'.
(2) Vaginal discharge, which may vary from scanty to profuse, is often grey or grey-green in colour, and tends to be homogeneous, watery, and often frothy. There is not usually any vulval soreness or irritation.
(3) The pH is 5 or greater and the amine test is positive.

Address for reprints: Dr A Blackwell, Department of Genitourinary Medicine, St Thomas's Hospital, London SE1 7EH

Accepted for publication 19 July 1982

(4) Clue cells are usually present in both wet mount preparation and Gram-stained slides.
(5) Lactobacilli are scanty or absent.
(6) Polymorphonuclear leucoytes are rare on both wet mount and stained preparations.
(7) Anaerobic bacteria (usually in association with *G vaginalis*) can be found with appropriate culture techniques.

STEPS IN DIAGNOSIS

History-taking

Although patients commonly complain of a smelly discharge anaerobic vaginosis can be a condition of minimal symptoms and signs. The patient may notice that symptoms vary with the stage of the menstrual cycle and complain that the menstrual blood has an abnormal odour. The character of the discharge may also vary at different times in the cycle, the progesterone-dominated latter part, for instance, giving rise to thicker, less frothy vaginal secretions. This sequence of events can give the patient and the examining doctor a false impression of spontaneous cure. It is therefore important to note the date of the last menstrual period, contraceptive method used (if any), and to ask whether symptoms alter in relation to the cycle.

The odour associated with this syndrome is often worse after sexual intercourse. Chen *et al* [6] suggested that the relatively alkaline semen reacted with the woman's infected vaginal secretions to release volatile amines. The physician should ask if the odour is worse after intercourse as this is a helpful sign.

Clinical examination

The discharge associated with anaerobic vaginosis is watery and non-irritant. The presence of a greyish discharge at the introitus is suggestive of the diagnosis, particularly if there is no vulval erythema. Some women with this condition have a vaginal discharge which is less than normal. If this is not a result of vaginal douching, it is a relevant clinical sign. More commonly, the patient will have a grey discharge which is homogeneous, often frothy, and tends to stick to the vaginal wall in an evenly spread coat. The vaginal mucosa has a normal appearance after the discharge has been swabbed away.

The presence or absence of vaginal odour should be noted. This can be identified either by sniffing the speculum after withdrawal (as suggested by Gardner and Dukes[7]) or by carrying out the sensitive amine test.

CLINICAL INVESTIGATIONS

pH measurement

The pH of the vagina in anaerobic vaginosis is $\geqslant 5$,[8] that is, less acid than normal vaginal secretions. We find narrow range (4-6) pH paper suitable. Since menstrual fluid and cervical mucus tend to raise the pH both these factors should be borne in mind when the findings are assessed. A short piece of pH paper can be clipped on to forceps and dipped into the vaginal discharge, or the paper may be placed in contact with secretions on the speculum after it has been withdrawn.

Amine test

This test, first described by Pheifer *et al*,[8] involves mixing the suspect vaginal discharge with an alkali, potassium hydroxide. It is both sensitive and specific if carried out as shown in fig 1. The test result is positive if an ammoniacal odour is released when potassium hydroxide is mixed with the discharge. It works equally well with secretion which has been left to dry on a microscope slide and, once appreciated, is subsequently easily recognised. The release of the odour is transient—hence the need to mix directly under the nose. Positive amine test results are also found in most patients harbouring *Trichomonas vaginalis* owing to the concomitant anaerobic component of the discharge. In agreement with K K Holmes (personal communication, 1982) we occasionally find a weakly positive amine test result when spermatozoa are present in the healthy vagina.

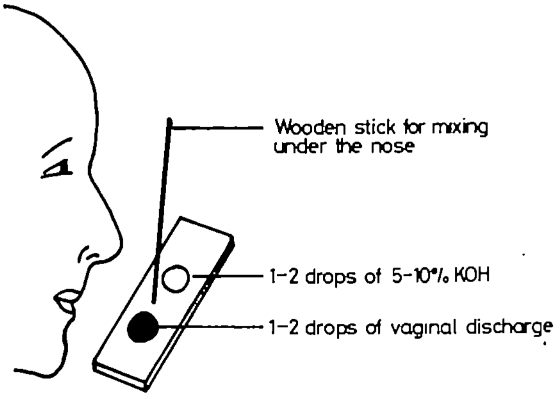

Wooden stick for mixing under the nose
1–2 drops of 5–10% KOH
1–2 drops of vaginal discharge

FIG 1 *Amine test*

Microscopy

The wet mount preparation, which is examined initially with the ×40 objective, consists of a drop of vaginal discharge mixed with a drop of normal saline and topped with a coverslip. Although not common practice it is possible to use the ×100 oil immersion objective with a drop of oil on top of the coverslip. This is of particular help in identifying vibrios and will occasionally show unexpected trichomonads which, if in small numbers, may be missed by low-power examination. The Gram-stained slide, which is routinely examined using the high-power objective for the presence of *Candida albicans,* will also show vaginal epithelial cells and any associated bacteria. The wet mount and stained slide together give information on (*a*) clue cells; (*b*) leucocytes; (*c*) lactobacilli; (*d*) vibrios; and (*e*) spermatozoa.

Clue cells—These vaginal epithelial cells with bacteria adhering to their surface are the hallmark of anaerobic vaginosis. They have a characteristic appearance on the wet mount (figs 2 and 3). The cell has a granular appearance with bacteria blurring the epithelial cell margins. The Gram-stained slide (fig 4) shows that these bacteria are chiefly Gram-negative or Gram-variable cocco-bacilli. Scattered Gram-positive organisms may also be seen but Döderlein's Gram-positive lactobacilli are conspicuous by their absence. In a recent series of 40 patients with anaerobic vaginosis initially diagnosed on history and clinical examination clue cells and a positive amine test result were found in all cases and each diagnosis was confirmed by subsequent culture of *G vaginalis* and anaerobes (Blackwell *et al*, unpublished data).

False-positive clue cells—In some cases lactobacilli stick to desquamated vaginal epithelial cells. If the wet mount alone is examined the doctor may be misled into a diagnosis of anaerobic vaginosis. The pH is low, however, the amine test result negative, and the stained slide shows the organisms to be Gram-positive rods (fig 5).

False-negative clue cells—Occasionally a patient with a florid, malodorous vaginal discharge of high pH will have no clue cells on the wet mount. Levinson *et al*[9] suggested that some women, perhaps those chronically infected, produced local IgA which blocked attachment of bacteria to the cell wall. In such cases the stained slide will show masses of Gram-negative to Gram-variable cocco-bacilli, most of which do not adhere to the cell wall. The pH and amine test are as for anaerobic vaginosis.

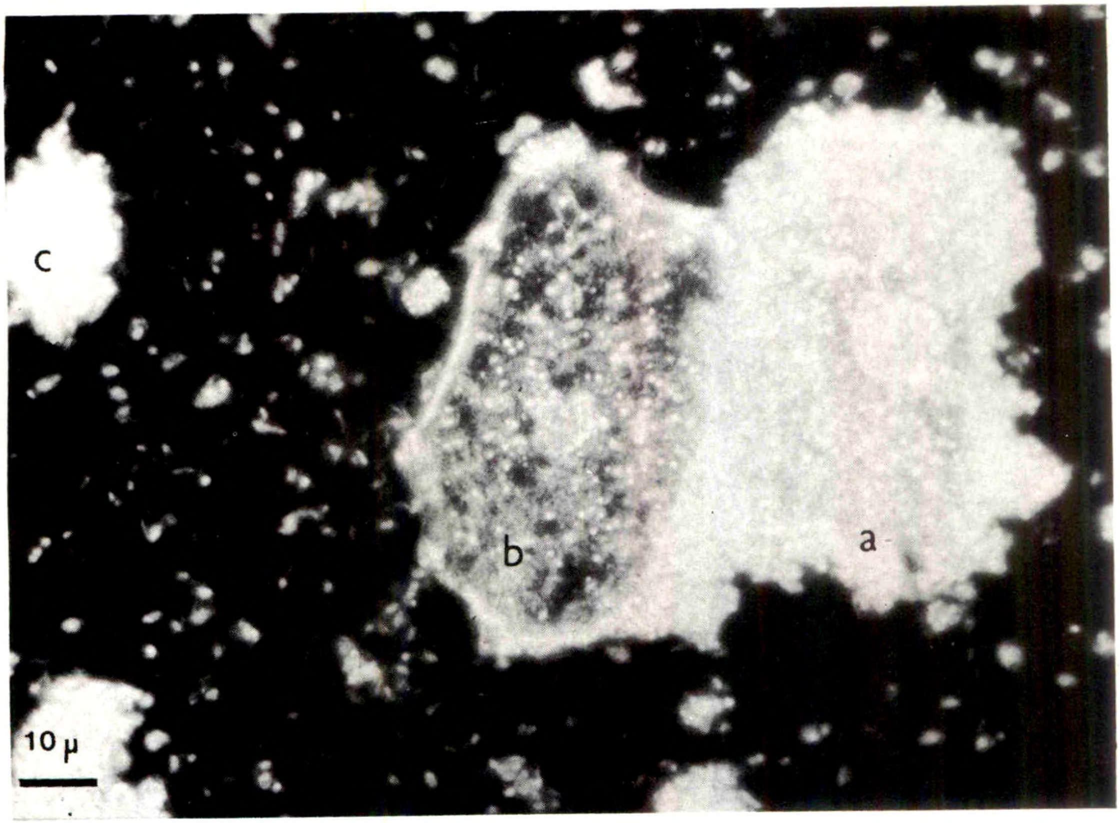


FIG 2 *Clue cell as seen using darkfield condenser. Note the presence of a relatively normal cell (b) immediately adjacent to the clue cell (a). Rafts of bacteria (c) are present in the surrounding medium together with numerous curved vibrios.*

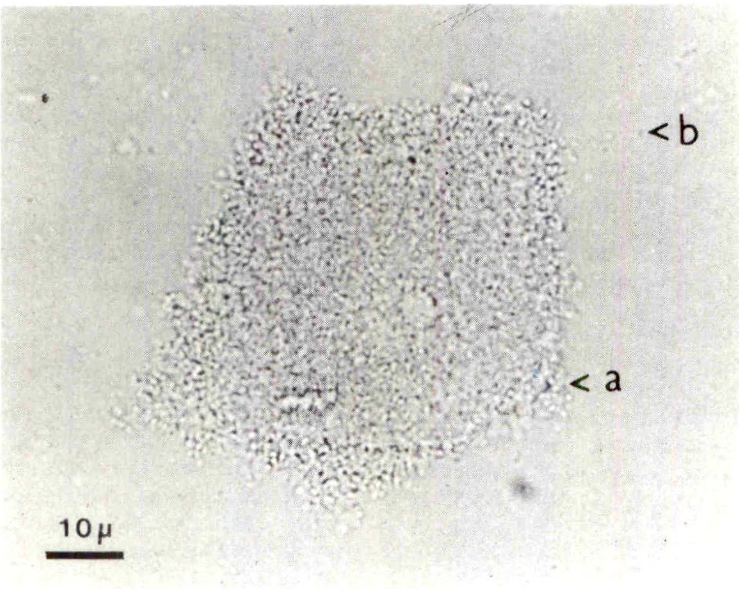


FIG 3 *Clue cell as seen using × 10 eye-piece and × 100 oil immersion objective under direct light microscopy. Note the blurred cell outline (a) and circular artifacts (b) produced by corkscrew movement of bacteria during exposure.*

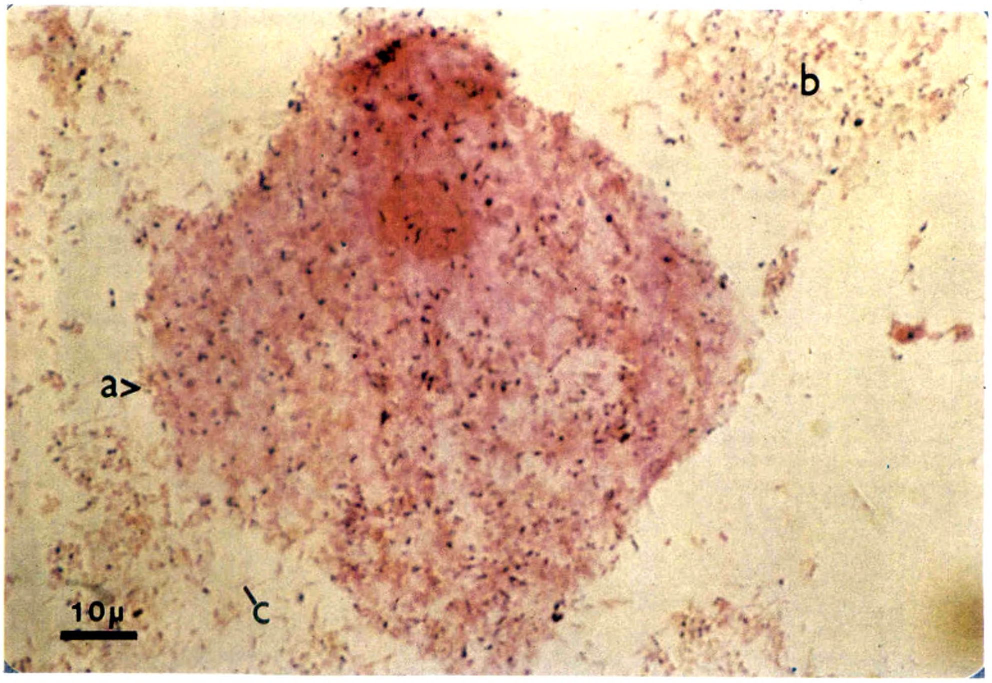


FIG 4 *Gram-stained preparation of a clue cell (a) showing adherent bacteria which are chiefly Gram-negative cocco-bacilli. Rafts of bacteria (b) are seen around the clue cell and Gram-negative curved bacilli (c) can be distinguished (anaerobic vibrios).*

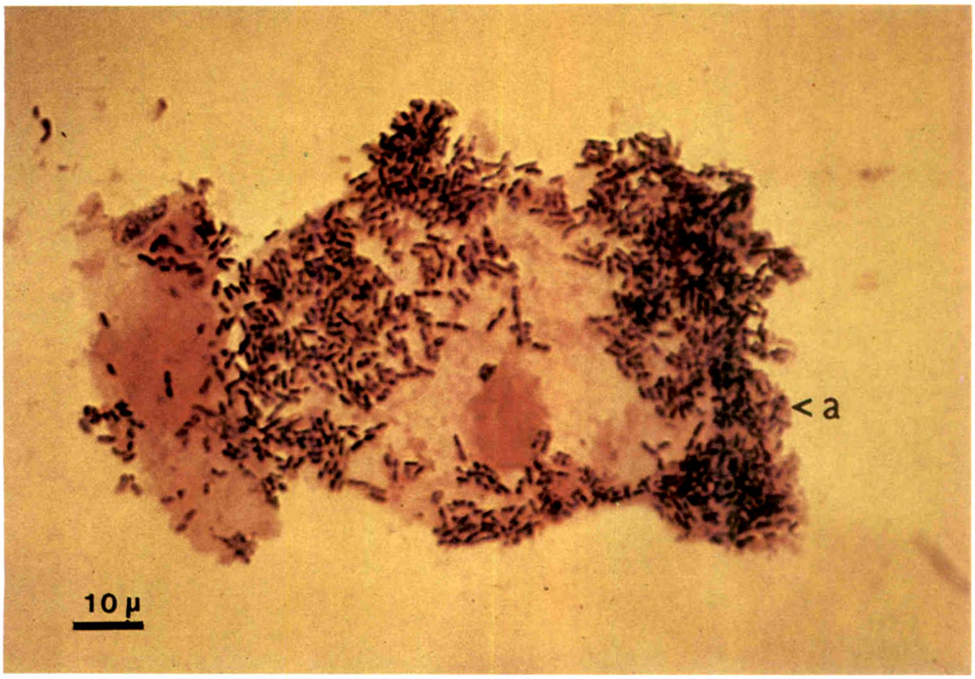


FIG 5 *A Gram-positive false clue cell in which the vaginal epithelial cell (a) is covered by Gram-positive lactobacilli which are part of the normal vaginal flora. On wet mount preparation these vaginal cells are easily mistaken for true clue cells.*

Polymorphonuclear leucocytes—Large numbers of leucocytes are rarely found in anaerobic vaginosis. If present the possibility of a coincidental chlamydial, gonococcal, or trichomonal infection should be suspected (see below).

Vibrios—There have been reports recently of an association of vibrios with non-specific vaginitis.[3 10 11] Examination of the wet preparation with the × 100 objective shows that these organisms are of two types. The larger, some 3μ in length, has a distinctive motion (not unlike that of a spermatozoon) and shows as a Gram-negative crescent on the Gram-stained slide. The smaller vibrio is one-third the size and is Gram-variable (figs 3 and 4). It is important to distinguish between vibrios and the effect of Brownian motion on other bacteria.

Spermatozoa—The presence of spermatozoa may explain the presence of a watery, grey, slightly malodorous discharge in an asymptomatic patient without clue cells. They may also help to distinguish between treatment failures and reinfections.

TREATMENT

In 1978 Pheifer *et al*[8] published results of an unblinded randomised trial which suggested that metronidazole was the most effective treatment for non-specific vaginitis. Since 1980, when our preliminary results concerning the bacteriology of the condition confirmed the importance of anaerobes, we have used metronidazole (400 mg twice daily for five days) to treat the condition. Early results of a single dose of 2 g metronidazole[4] indicate that this regimen, which is satisfactory in the treatment of *T vaginalis,* is inadequate for anaerobic vaginosis.

ASSESSMENT OF CURE

The disappearance of clue cells from the wet mount preparation after treatment should be enough to confirm therapeutic success; there is also a dramatic change in the appearance of the Gram-stained slide (figs 4 and 6). The absence of Gram-negative bacteria and their replacement by lactobacilli is evidence of bacteriological cure and correlates well with symptomatic relief.

MIXED INFECTIONS

Anaerobic vaginosis is often found in conjunction with other sexually transmitted diseases. In such cases there may be an alteration in clinical symptoms, signs, and microscopical findings.

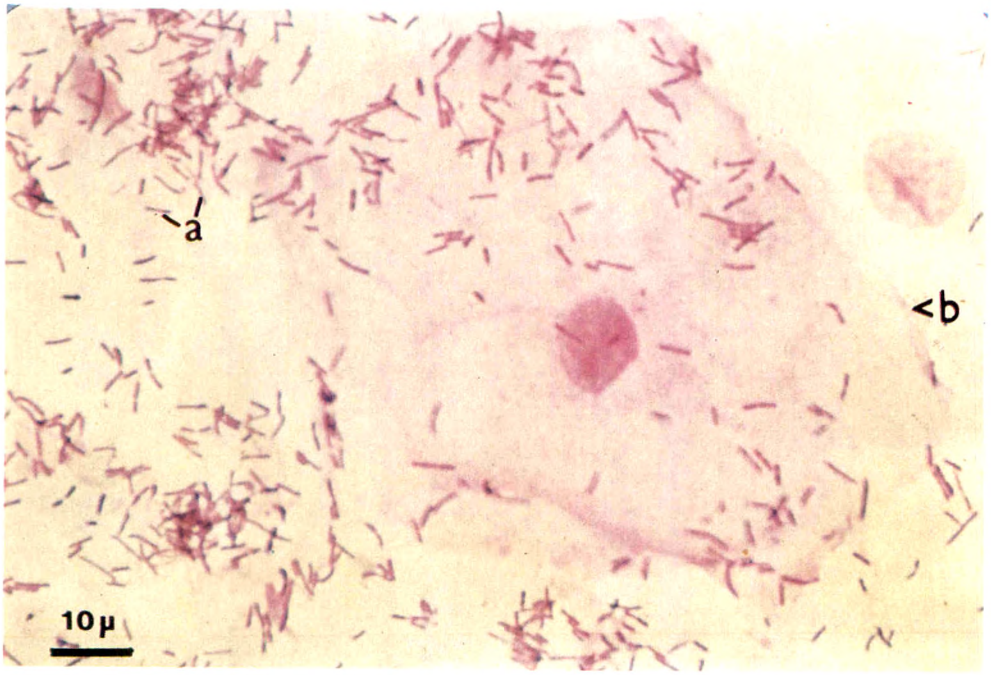


FIG 6 *Gram-stained specimen of normal vaginal secretions. This specimen was taken from the same patient as in fig 3 after successful treatment with metronidazole. Note that the bacteria present (a) are of uniform appearance (lactobacilli) and that the vaginal epithelial cell outline (b) is easily distinguished.*

Of other vaginal infections, *C albicans* is not often found with anaerobic vaginosis. When the two infections do coincide, however, there may be soreness and irritation and examination shows candidal plaques floating in the foul-smelling anaerobic discharge. The amine test result will be positive but the pH may give atypical results. Microscopy should show classical clue cells together with candidal spores or mycelia or both.

T vaginalis has an almost invariable anaerobic component to the discharge and *G vaginalis* is also found in most cases. Since the treatment for both conditions can be identical, the other findings become academic once trichomonads have been found.

Clue cells and an anaerobic discharge are frequently found in the presence of gonorrhoea. Of 200 consecutive cases of gonorrhoea in women attending St Thomas's Hospital early in 1982, 36% were found to have anaerobic vaginosis as against 22% with trichomoniasis. Likewise, 35% of women attending as sexual contacts of men with non-specific urethritis had clue cells. The discharge in these cases may not be typical but the pH is high, the amine test result is positive, and microscopy usually shows increased leucocytes in addition to clue cells.

Discussion

The spate of recent reports and review articles on non-specific vaginitis, *G vaginalis,* and anaerobic infection is evidence enough of the renewed interest in these conditions over the last few years. In spite of excitement about the sexually transmitted component in such bizarre conditions as Kaposi's sarcoma and *Pneumocystis carinii* pneumonitis, it is likely that for purely numerical reasons anaerobic vaginosis will be the sexually transmitted disease of the '80s.

Even having adopted the pragmatic approach advocated here in terms of diagnosis, there remain considerable problems in terms of management. Does one accept that anaerobic infection is always sexually transmitted? Should the sexual partners of affected women be treated? If so, should this be on the basis of a history of contact alone or should attempts be made to establish evidence of infection in the male partner? There is a pressing need for research designed to answer these questions.

Our early experience of examining male contacts of women with anaerobic vaginosis gives some pointers. In the absence of attempts to culture anaerobes we have found *G vaginalis* in the urethra of some 30% of male contacts and would expect this figure to increase as sampling techniques improve. Perhaps more interestingly, almost 40% of such contacts, although asymptomatic, satisfy our microscopical criteria for the diagnosis of non-gonococcal urethritis (Kohiyar, unpublished observations). These patients are chlamydia-negative. If anaerobic culture techniques were applied with as much vigour to the male urethra as they have been in the past to the vagina some light might possibly be thrown on cases of chlamydia-negative urethritis.

We would like to express our thanks to Miss E Taylor, Alan Fox and Professor I Phillips, Department of Microbiology, and T Brandon, Department of Medical Photography, St Thomas's Hospital, and to May and Baker for financial support.

References

1. Taylor E, Blackwell A, Barlow D, Phillips I. *Gardnerella vaginalis,* anaerobes and vaginal discharge. *Lancet* 1982; i: 1376-9.
2. Taylor E. The vaginal flora in health and disease. *MSc thesis,* 1981. University of Surrey.
3. Taylor E, Phillips I. Anaerobic curved rods in vaginitis. *Lancet* 1982;i:221.
4. Blackwell A, Fox A, Phillips I, Barlow D. Metronidazole in treatment of non-specific vaginitis. Clinical and microbiological findings in 10 patients given 2 grams of metronidazole. *Scand J Infect Dis* 1982 (in press).
5. Speigel CA, Eschenbach D, Schoenknecht F, Holmes KK Anaerobic bacteria in non-specific vaginitis. *N Engl J Med* 1980;**303**:601-7.
6. Chen KCS, Forsyth PS, Buchanan TM, Holmes KK. Amine content of vaginal fluid from untreated and treated patients with non-specific vaginitis. *J Clin Invest* 1979;**63**:828-35.
7. Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified "non-specific vaginitis". *Am J Obstet Gynecol* 1955;**69**:962-76.
8. Pheifer TA, Forsyth PS, Durfee MA, Pollock HM, Holmes KK. Non-specific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. *N Engl J Med* 1978; **298**:1429-34.
9. Levison ME, Trestman I, Quach R, Sladowski C, Floro CN. Quantitative bacteriology of the vaginal flora in vaginitis. *Am J Obstet Gynecol* 1979;**133**:139-44.
10. Hjelm F, Forsum U, Wallin J. Anaerobic curved rods in vaginitis. *Lancet* 1982;i:1353-4.
11. Skarin A, Mårdh P-A. Comma-shaped bacteria associated with vaginitis. *Lancet* 1982;i:342-3.

Br J Vener Dis 1982;58:394-9

Metronidazole resistance of Trichomonas vaginalis as a cause of treatment failure in trichomoniasis

A case report

JAROSLAV KULDA, MILENA VOJTĚCHOVSKÁ, JAN TACHEZY, PAVOL DEMEŠ, AND EVA KUNZOVÁ

From the Department of Parasitology, Charles University, Prague; and the Laboratory of Clinical Parasitology, University Hospital 1, Prague, Czechoslovakia

SUMMARY Six isolates of a strain (MRP-MT) of *Trichomonas vaginalis* obtained from a woman before and after unsuccessful treatment with metronidazole had an appreciably lower susceptibility to metronidazole both in vitro in the aerobic tube assay and in vivo in the mouse assay than did control strains from patients cured with standard doses of the drug.

Our results support recent evidence that metronidazole-resistant strains of *T vaginalis* do cause treatment failure. Resistance of these strains could be detected in vitro under only aerobic but not anaerobic conditions. The prevalence of metronidazole-resistant strains of *T vaginalis* should be kept under surveillance in order to estimate their clinical importance.

The patient harbouring the resistant strain MRP-MT was finally cured with increased doses of ornidazole.

Introduction

Resistance of *Trichomonas vaginalis* to 5-nitroimidazole drugs has been a matter of controversy.[1] Although drug resistance of trichomonads has not been shown convincingly in vitro there have been clinical reports of treatment failures. The possibility of the existence of metronidazole-resistant strains of *T vaginalis* has been met with considerable scepticism.[2-4] Persistent trichomonal infections which could not be ascribed to reinfection were attributed to altered pharmacokinetics in patients[5] or to a lowering of the drug concentration in the vagina by concomitant bacteria.[4 6 7] Recently, however, a decreased susceptibility to metronidazole has been proved unequivocally in several strains of *T vaginalis* isolated from patients who were refractory to treatment.[8-10]

Meingassner *et al*[11] showed the importance of assay conditions in the in-vitro detection of resistance of trichomonads to 5-nitroimidazoles. Using a laboratory-developed drug-resistant strain of the cattle parasite *Tritrichomonas foetus*[12] these workers found that the resistance of this strain could be ascertained in vitro only under aerobic conditions. Aerobic assay systems have been developed to allow reliable determinations of trichomonad susceptibility to 5-nitroimidazoles in vitro.[8 10]

We report metronidazole resistance in a strain of *T vaginalis* isolated from a female patient who did not respond to several courses of treatment with 5-nitroimidazoles.[13]

Patient and methods

CASE REPORT

The patient was a 41-year-old woman who gave a four-year history of recurrent vulvo-vaginitis caused by *T vaginalis*. During this period she had been treated unsuccessfully seven times with metronidazole (Entizole, Polfa) and once with tinidazole (Fasigyn, Pfizer). In five of the treatment courses metronidazole (250 mg) was taken orally twice daily supplemented with 500 mg inserted vaginally once daily for 10 days. In one course the daily dose of metronidazole was increased to 1 g, and in another five 250-mg oral tablets (a total of 1250 mg metronidazole) were taken daily for three days.

Address for reprints: Dr J Kulda, Department of Parasitology, Charles University, 128 44 Prague 2, Viničná 7, Czechoslovakia

Accepted for publication 17 July 1982

Tinidazole was given orally in 150 mg tablets twice daily for seven days.

When she was examined in our department the patient had an acute vulvo-vaginitis with an inflammatory discharge containing many trichomonads (isolate MRP-MT-1). She was given two successive courses of treatment with oral metronidazole (Flagyl, Specia) in a dosage of 500 mg twice daily for five days. Similar treatment was given to her husband. After completion of the treatment the symptoms disappeared and no trichomonads were found in a wet mount preparation. *T vaginalis* was cultured), however, from vaginal material (isolate MRP-MT-3). Within a month the symptoms had returned and numerous trichomonads were detected in the vaginal exudate.

To determine whether the treatment failure was due to poor absorption of metronidazole the patient was admitted to hospital and given metronidazole in a single oral dose of 2 g. The serum concentrations of the drug were determined two, four, and 24 hours after treatment. Satisfactory concentrations of metronidazole were present in the serum (table I). The treatment was completed by giving the patient metronidazole in a dosage of 1g twice daily for seven days. Despite symptomatic relief and the reappearance in the vagina of *Lactobacillus vaginalis* trichomonads were cultured from vaginal material (MRP-MT-8). The patient was eventually cured with ornidazole (Tiberal, Roche) 1 g orally twice daily supplemented with 500 mg inserted vaginally once daily for six days. The symptoms disappeared completely and *T vaginalis* could not be cultured from vaginal material.

TABLE I *Serum concentrations of metronidazole after administration of a single oral dose of 2 g (polarographic determinations at −0·7 V)*

Hours after administration of drug	*Serum metronidazole concentration (µg/ml)*
4	33
8	25
24	12

ISOLATION AND CULTIVATION OF *T VAGINALIS*

The metronidazole-resistant strain under study, *T vaginalis* MRP-MT, was isolated from the vagina of the patient on several occasions before and after unsuccessful treatment. A total of eight isolates were obtained during the two-year study of the patient. Six of these isolates were assayed for susceptibility to metronidazole in vitro.

Eight strains of *T vaginalis* which were susceptible to metronidazole (TV5-27, TV7-37, TV10-02, TV14-85, TV17-48, TV67-77, TV87-64, and TV99-51) were isolated from patients who had attended our clinic. All patients had been successfully treated with a single course of oral metronidazole.

Trichomonads were isolated from vaginal secretions collected with a cotton swab from the posterior fornix of the vagina. Cultures were established and maintained in Diamond's (trypticase, yeast extract, maltose) medium (TYM),[14] pH 6·0 with trypticase (BBL) replaced by tryptone (Oxoid, L-42). The medium was supplemented with 10% heat-inactivated horse serum. Concomitant micro-organisms were eliminated by migration in the DeCarneri tube[15] and with the aid of antibiotics. All isolates were treated three times with kanamycin sulphate (2500 µg/ml), three times with penicillin G (5000, 2000 and 1000 IU/ml) and once with streptomycin sulphate (1000 µg/ml). The absence of contaminating micro-organisms was confirmed by standard sterility tests including assays for *Mycoplasma* spp. Axenised cultures with the addition of 5% dimethylsulphoxide were stored in liquid nitrogen.

The two reference metronidazole-resistant strains, obtained by courtesy of Dr J G Meingassner, Sandoz Forschungsinstitut, Vienna, were *T vaginalis* IR-78 from Vienna, Austria,[8] and *T vaginalis* "Fall River" from the USA.[10] The former was a fresh subculture of the original stabilate,[8] and the latter had been maintained for several months by serial transfers. Both strains had been grown axenically. After arrival at our laboratory the strains were transferred to TYM medium and cryopreserved.

Cultures used in the experiments were derived from frozen stabilates and grown in TYM medium without antibiotics. Except for the "Fall River" strain, none of the strains was maintained in active culture at 37°C for longer than three weeks.

SUSCEPTIBILITY ASSAYS

Susceptibility of trichomonads to metronidazole in vitro was estimated as a minimal lethal concentration (MLC) of the drug using an aerobic tube assay. Suspensions of trichomonads (1×10^5 cells/ml) in 1 ml of the aerobic medium (TYM without cysteine and ascorbic acid) were exposed for 48 hours to twofold serial dilutions of metronidazole, and the viability of the parasites was assayed by subsequent cultivation in a drug-free medium. The assay was performed in 100 × 15 mm centrifuge tubes, each containing 0·9 ml of a suspension of parasites in the aerobic TYM and 0·1 ml of the metronidazole solution. The tubes were closed with metal caps allowing equilibration with air. After incubation at 37°C for 48 hours the cells were centrifuged ($1600 \times g$), washed in saline, and resuspended in 5 ml of standard TYM. The metal caps were replaced by non-toxic rubber stoppers and the tubes incubated at 37°C for seven days. The MLC at 48 hours (MLC_{48h}) was defined as the lowest concentration of metronidazole at which no viable trichomonads could be detected by cultivation. The assays were run in duplicate and repeated at least twice; additional dilutions were included to refine endpoints.

The in-vitro susceptibility to metronidazole was assayed also under anaerobic conditions in 13 × 100 mm screw-cap tubes (Bellco). Each tube contained 4·4 ml of standard TYM (with reducing compounds included), 0·1 ml of metronidazole solution, and 0·5 ml of trichomonad suspension (to make a final concentration of 1×10^5 cells/ml). After 48 hours exposure to metronidazole the cells were washed with saline and resuspended in a drug-free medium.

The in-vivo susceptibility to metronidazole was assayed by estimating the DC_{50} (dose needed to cure 50% of infections) using the subcutaneous mouse assay described by Meingassner and Thurner.[8] Mice were inoculated subcutaneously in each flank with trichomonads which were in the logarithmic phase of growth. Metronidazole was given orally at two, 18, and 24 hours after inoculation. The therapeutic effect was evaluated on day 6 by determining whether viable parasites were present or absent at the site of inoculation (by microscopy and culture). Male albino SPF mice (18-20 g) supplied by Velaz (Prague, Czechoslovakia) were used in preliminary experiments performed in Prague. Thirty mice were inoculated with *T vaginalis* isolate MRP-MT-3 and 30 received the susceptible control strain TV17-48. The inocula contained 1×10^6 cells in 0·5 ml of TYM medium. Groups of six mice were given the same dose of metronidazole. Further experiments on the metronidazole-resistant isolate MRP-MT-3 were performed by J G Meingassner in Vienna. Female NMRI mice (weight 10-12 g), supplied by Gassner (Salzberg, FRG) were inoculated subcutaneously and intraperitoneally. A total of 55 mice (two experiments) were inoculated subcutaneously (inocula 4×10^5 cells) and 118 mice (three experiments) intraperitoneally ($1 \cdot 2 \times 10^6$ cells). The same dose of the drug was given to groups of seven or eight mice. Untreated controls were included in the experiments. Curative doses were determined by means of a probit analysis.

METRONIDAZOLE

Pure metronidazole produced by Specia or Bayer was used throughout the experiments. For in-vitro assays stock solutions were prepared in glass-distilled water (50 mg/10 ml), and the substance was dissolved by autoclaving. Serial dilutions were made in aerobic TYM. For mouse assays the drug was dissolved and administered in a 10% aqueous solution of dimethylsulphoxide and 0·2% Tween 80 (v/v).

SERUM CONCENTRATIONS OF METRONIDAZOLE

Serum concentrations of metronidazole were determined polargraphically by the method of Kane[16] using an LP-60 polarograph (Laboratory Supplies, Prague) at −0·7 V. Before the measurement the sera were diluted 1/10 with potassium chloride (final KCl concentration 0·001 mol/l).

Results

The data summarised in table II showed notable differences between the in-vitro susceptibility to metronidazole of MRP-MT isolates and that of control *T vaginalis* strains from patients responding favourably to a standard treatment. In an aerobic tube assay the mean MLC_{48h} (81 μg/ml) for the MRP-MT isolates was about 29 times higher than that for the control strains (TV) (2·8 μg/ml). The MLCs for the MRP-MT isolates were within the range of those for the reference metronidazole-resistant strains from Austria (IR-78) and the USA ("Fall River"). The resistance in vitro was detectable only in the aerobic assay. Under anaerobic conditions all assayed strains were susceptible to metronidazole with a mean MLC_{48h} of 2·15 μg/ml.

When the efficacy of metronidazole against infection with the isolate MRP-MT-3 was further assayed in vivo in subcutaneously and intraperitoneally infected mice the susceptibility of this isolate to metronidazole was low (table III). Its relative drug resistance was more pronounced in mice which had been inoculated subcutaneously. The DC_{50} value of the MRP-MT-3 isolate, calculated from the subcutaneous assays performed in Prague, was about 20 times higher than that of the drug-susceptible control TV 14-85. The highest dose given (3 × 800 mg/kg) was insufficient to obtain a 100% cure. In Meingassner's experiments none of the mice infected subcutaneously was cured with the highest dose assayed (3 × 400 mg/kg). Intraperitoneal infections with the MRP-MT-3 isolate were successfully cured with metronidazole. The doses necessary for eliminating the infection, however, were relatively high (3 × 400 mg/kg) and the DC_{50} values about eight times higher than that for the Viennese control strain E (table III).

The MLC values for six isolates of the MRP-MT strain (table II) showed that throughout the two years of study the protozoon did not respond to repeated treatment by an increase in resistance.

TABLE II *In-vitro susceptibility to metronidazole of T vaginalis MRP-MT isolates compared with reference metronidazole-resistant strains (IR-78 and Fall River) and eight drug-susceptible strains*

	MLC$_{48}$ of metronidazole (μg/ml)*			
	Aerobic assay			
T vaginalis strain	*Mean*	*n*†	*Range*	*Anaerobic assay*
Resistant strains:				
MRP-MT-1	110	6	80-150	2·0
MRP-MT-2	65	4	40-100	2·5
MRP-MT-3	85	4	80-100	2·5
MRP-MT-5	80	4	80	2·0
MRP-MT-7	60	2	40-80	ND
MRP-MT-8	87	6	80-100	2·5
IR-78	52	8	40-100	(4·0)‡
Fall River	80	3	80	(2·0)‡
Susceptible strains:				
TV 5-27	1·25	2	1·25	ND
TV 7-37	5·0	2	5·0	ND
TV 10-02	2·0	4	1·25 – 2·5	ND
TV 14-85	5·6	8	5·0 – 10·0	2·0
TV 17 – 48	2·5	2	2·5	ND
TV 67-77	2·2	4	1·25 – 2·5	1.0
TV 87 – 64	2·5	2	2·5	1.0
TV 99-51	1·25	2	1·25	ND

*Minimal lethal concentration of metronidazole after 48 h exposure of parasites to the drug
†Number of determinations
‡Data from reference[10] obtained by anaerobic tray test
ND = not determined

TABLE III *In-vivo susceptibility to metronidazole of the T vaginalis isolate MRP-MT-3 compared with the drug-susceptible strains TV 14-85 and E*

		Subcutaneous assay		*Intraperitoneal assay*	
Strain	*Assayed by*	*No of mice*	*DC$_{50}$ (mg/kg)(×3)**	*No of mice*	*DC$_{50}$ (mg/kg)(×3)*
MRP-MT-3	Kulda *et al*	30	300		
	Meingassner	55	>400	94	115·9
TV 14-85	Kulda *et al*	30	15		
E†	Meingassner		11·2		14·7

*Dosage of metronidazole given orally at two, 18, and 24 h
†Data on this strain is given in reference[8]

Discussion

Our results showed a relative resistance to metronidazole of the *T vaginalis* strain MRP-MT isolated in Prague from a patient with a resistant vulvo-vaginitis. Neither reinfection nor failure to take the prescribed doses of the drug could be regarded as being responsible for the persistent infection, because one of the unsuccessful courses of treatment was given while she was in hospital. Serum concentrations of metronidazole after oral administration indicated satisfactory absorption of the drug from the gut. The resistance of the trichomonads isolated from the patient was shown in laboratory assays under bacteria-free conditions, thereby excluding any interference by bacteria in the trichomonocidal ability of the drug. The low susceptibility of the MRP-MT strain to metronidazole was detected in vitro by an aerobic tube assay as well as in vivo by determining the CD$_{50}$ in mice infected subcutaneously and intraperitoneally. The reliability of the laboratory tests was controlled by assaying in parallel the MRP-MT isolates, the reference metronidazole-resistant strains, and drug-susceptible strains isolated from patients cured successfully with standard doses of metronidazole. These data indicated that infection with a metronidazole-resistant strain was the primary cause of the treatment failure in our patient.

The properties of the MRP-MT strain were similar to those described recently by other workers.[8 10 17] All these strains caused treatment-resistant diseases in man; their resistance was detected in vivo in subcutaneously infected mice and also in vitro if assay conditions were aerobic. The importance of using an aerobic system for the in-vitro demonstration of resistance was confirmed by others.[18] Forsgren and Forssman[9] identified their metronidazole-resistant strain using a tube assay which appeared to be essentially aerobic. On the other hand, the relatively low minimum inhibitory concentrations (MICs) reported for strains of *T vaginalis* from treatment failures in Britain (MIC 20-25 μg/ml) might have been accounted for by the use of an assay system in which there is a lower availability of oxygen.[19 20]

The need for aerobic conditions to show resistance in an anaerobic organism seems to be an unusual phenomenon. Persisting doubts on the real existence of metronidazole-resistant strains of *T vaginalis* are therefore understandable. Incomplete anaerobiosis was the cause of a false metronidazole "resistance" in anaerobic bacteria.[21] Even in trichomonads the presence of air suppressed the uptake of metronidazole[22] and increased MLC values of the drug.[11] Recently published comparative data[8-10] have shown differences in aerobic MLC values between *T vaginalis* strains isolated from readily curable trichomoniasis and from a treatment-resistant infection. This evidence is supported by the results of our study.

Laboratory experiments indicated that the resistance of *T vaginalis* to metronidazole involves an aerobic tolerance to the drug. Implications of this phenomenon for the drug-parasite interaction in situ need to be clarified. The number of resistant trichomonads in the vagina is appreciably reduced after treatment with metronidazole. Probably very few potentially resistant trichomonads meet with conditions in the human vagina which favour their aerobic drug-tolerance. Data on oxygen tension and redox potential in different parts of the infected vagina would clarify the physiological background of infections with metronidazole-resistant trichomonads. Biochemical mechanisms of *T vaginalis* resistance to metronidazole are not yet known.[23 24] There is some evidence, however, that this aerobic type of resistance is not connected with enzyme deficiences eliminating the pathway of anaerobic pyruvate metabolism essential for activation of 5-nitroimidazoles. Such enzyme lesions have been observed in metronidazole-resistant mutants of *Tritrichomonas foetus*[25 26] and *Bacteroides fragilis*[27] but not in the drug-tolerant *T vaginalis*.[24 28 29]

Metronidazole resistance of the strain of *T vaginalis* MRP-MT which was reisolated repeatedly from the vagina of our patient did not increase as a result of three unsuccessful courses of treatment; each of the courses apparently exerted considerable selection pressure on the trichomonads. Similarly, increase in resistance was not observed in a strain of *T vaginalis* isolated in Sweden after treatment with tinidazole.[9] In both cases, however, the patients had been treated with nitroimidazoles before initial isolation of the parasite. It is uncertain whether the resistance was induced by inadequate treatment or was intrinsic to the infecting organism.

Recent evidence indicates that metronidazole-resistant strains of *T vaginalis* cannot be regarded as unique in their incidence. Isolation of resistant strains from patients have been reported in Austria,[8] Sweden,[9] Britain,[19 20] and the USA.[10 17 18] This calls for a systematic follow-up of treatment failures and extensive surveys of a random population to establish the prevalence of resistant strains. Until then it would be premature to draw conclusions on the clinical importance of metronidazole resistance in strains of *T vaginalis*. In view of the absence of clinically available drugs which could replace 5-nitroimidazoles, however, the resistance of *T vaginalis* to metronidazole should be followed with concern.

We wish to thank Dr J G Meingassner (Sandoz Forschungsinstitut, Vienna, Austria) for assaying the in-vivo efficacy of metronidazole against our *T vaginalis* isolate and for his generous permission to publish his data in this paper. We also appreciate Dr Meingassner's help in providing the reference metronidazole-resistant strains of *T vaginalis*. Our sincere thanks are due to Dr M Müller (Rockefeller University, New York, USA) for his kind permission to use his metronidazole-resistant strain and for sending us reprints of his papers, to Dr J Čerkasov (Charles University, Prague, Czechoslovakia) for his valuable advice on a polarographic determination of metronidazole, and to our patient for her patience and co-operation.

References

1. Honigberg BM. Trichomonads of importance in human medicine. In: Kreier JP, ed. *Parasitic Protozoa*, vol II. New York: Academic Press, 1978:275-454.
2. Korner B, Jensen HK. Sensitivity of *Trichomonas vaginalis* to metronidazole, tinidazole, and nifuratel in vitro. *Br J Vener Dis* 1976;52:404-8.
3. Roe FJC. Metronidazole: review of its uses and toxicity. *J Antimicrob Chemother* 1977;3:205-12
4. Edwards DI. Mechanisms of selective toxicity of metronidazole and other nitroimidazole drugs. *Br J Vener Dis* 1980;56:285-90.
5. Kane PO, McFadzean JA, Squires S. Absorption and excretion of metronidazole. II Studies on primary failures. *Br J Vener Dis* 1961;37:276-7.
6. Ralph ED, Clarke DA. Inactivation of metronidazole by anaerobic and aerobic bacteria. *Antimicrob Agents Chemother* 1978;14:377-83.
7. Edwards DI, Thompson EJ, Tomusange J, Shanson D. Inactivation of metronidazole by aerobic organisms. *J Antimicrob Chemother* 1979;5:315-6.
8. Meingassner JG, Thurner J. Strain of *Trichomonas vaginalis* resistant to metronidazole and other 5-nitroimidazoles. *Antimicrob Agents Chemother* 1979;15:254-7.
9. Forsgren A, Forssman L. Metronidazole-resistant *Trichomonas vaginalis*. *Br J Vener Dis* 1979;55:351-3.

10. Müller M, Meingassner J, Miller WA, Ledger WJ. Three metronidazole-resistant strains of *Trichomonas vaginalis* from the United States. *Am J Obstet Gynecol* 1980;138:808-12.
11. Meingassner JG, Mieth H, Czok R, Lindmark DG, Müller M. Assay conditions and the demonstration of nitroimidazole resistance in *Trichomonas foetus*. *Antimicrob Agents Chemother* 1978;13:1-3.
12. Meingassner JG, Mieth H. Cross-resistance of trichomonads to 5-nitroimidazole-derivatives. *Experientia* 1976;32:183-4.
13. Kulda J, Demeš P, Vojtěchovská M, Kunzová E. Metronidazole resistant strain of *Trichomonas vaginalis* from a patient refractory to treatment. *J Protozool* 1980;27:70A.
14. Diamond LS. The establishment of various trichomonads of animals and man in axenic cultures. *J Parasitol* 1957;43:488-90.
15. DeCarneri I. Isolation of *Trichomonas vaginalis* from fungi and bacteria. *Am J Trop Med* 1956;5:210-2.
16. Kane PO. Polarographic methods for the determination of two anti-protozoal nitroimidazole derivatives in materials of biological and non-biological origin. *J Polarograf Soc* 1961;8:58-62.
17. Lossick JG. Metronidazole. *N Engl J Med* 1981;304:735.
18. Smith RF, DiDomenico A. Measuring the in-vitro susceptibility of *Trichomonas vaginalis* to metronidazole. *Sex Transm Dis* 1980;7:120-4.
19. Heyworth R, Simpson D, McNeillage GJC, Robertson DHH, Young H. Isolation of *Trichomonas vaginalis* resistant to metronidazole. *Lancet* 1980;ii:476-8.
20. Waitkins S, Thomas DJ. Isolation of *Trichomonas vaginalis* resistant to metronidazole. *Lancet* 1981;ii:590.
21. Milne SE, Stokes EJ, Waterworth PM. Incomplete anaerobiosis as a cause of metronidazole "resistance". *J Clin Pathol* 1978;31:933.
22. Müller M, Lindmark D. Uptake of metronidazole and its effect on viability in trichomonads and *Entamoeba invadens* under anaerobic and aerobic conditions. *Antimicrob Agents Chemother* 1976;9:696-700.
23. Müller M. Action of clinically utilized 5-nitroimidazoles on microorganisms. *Scand J Infect Dis* 1981;suppl 26:31-41.
24. Müller M. Resistance to 5-nitroimidazoles in pathogenic microorganisms. In: *Chemistry) Pharmacology and Clinical Application of Nitroimidazoles*. Proceedings of the International Conference, Cesenatico, Italy, 27-30 August 1980. Bologna, Italy: Universita Degli Studi di Bologna, in press.
25. Kulda J, Čerkasov J, Čerkasovová A. *Tritrichomonas foetus* resistant to metronidazole: development of resistance in vitro. In: *Progress in Protozoology*. Proceedings of the VI International Congress of Protozoology. Abstracts. Warsaw, Poland: 1981:203.
26. Čerkasovová A, Cerkasov J, Kulda J, Demes P. Metronidazole action on *Trichomonas foetus:* enzyme activities in the strains of a different resistance to the drug. In: Van den Bossche H, ed. *Host Invader Interplay*. Amsterdam, Netherlands: Elsevier/North-Holland Biomed Press, 1980:669-72.
27. Britz ML, Wilkinson RG. Isolation and properties of metronidazole-resistant mutants of *Bacteroides fragilis*. *Antimicrob Agents Chemother* 1979;16:19-27.
28. Müller M. Physiological studies on *Trichomonas vaginalis* with lowered susceptibility to metronidazole. *J Protozool* 1980;27:26A-27A.
29. Demes P. Biological properties of metronidazole-resistant trichomonads. *Thesis*. Prague, Czechoslovakia: Department of Parasitology, Charles University, 1980.

Br J Vener Dis 1982;**58**:400-1

Scissor excision of anogenital warts

JOHN M GOLLOCK,* KENNETH SLATFORD,† AND JENNIFER M HUNTER†
*From *Wards 9 and 10, and the †Department of Genitourinary Medicine, Royal Infirmary, Edinburgh*

SUMMARY Anogenital warts were treated by scissor excision in 34 patients while under general anaesthesia; most patients were referred by the department of genitourinary medicine. In 28 patients who were adequately followed up the primary success rate was 71·4% and the rate of recurrence of warts was 9·3%. The technique gave good cosmetic results with few complications.

Introduction

Several techniques have been described for the treatment of anogenital warts, but the results of these methods have not been accurately recorded. Although the results of immunotherapy[1] and cryotherapy[2] are well documented those of surgical techniques have not been detailed. Thomson and Grace,[3] however, described a simple method called scissor excision, which yielded good results. By this method saline-adrenaline solution (1/300 000) was infiltrated to separate the warts thereby allowing their accurate removal with scissors. The use of adrenaline also aids haemostasis. The aim of this study was to assess the merits of scissor excision in patients who presented initially to a department of genitourinary medicine.

Patients and methods

Most patients were referred for scissor excision of warts from the department of genitourinary medicine, Royal Infirmary, Edinburgh, between April 1980 and April 1982; the remainder were referred directly to the surgical unit. All patients were treated as inpatients. The procedure was performed under general anaesthesia with the patient in the lithotomy position. The method described by Thomson[4] was followed accurately and is summarised below.

The area bearing the warts was infiltrated with saline-adrenaline solution (1/300 000). The amount required ranged from 20 to 150 ml depending on the number and distribution of the warts. Once the area had blanched, the warts were removed with scissors by cutting at the base of the wart. Cutting was performed from the back of the wart to the front so that exudate and blood did not obscure progress.

Intra-anal warts were excised in a similar manner using a Park's anal retractor, the saline-adrenaline solution being injected into the submucous plane. If an area of confluent warts was present the block of mucosa was excised and the defect repaired with 3/0 Dexon sutures. Diathermy was not required for haemostasis. The wounds were dressed with gauze swabs soaked in a 1/20 Milton solution. Postoperative sedation was given as required.

On the day after the operation the dressings were removed and the wounds inspected; the decision for discharge was usually made then. The patients were followed up in the department of genitourinary medicine with surgical liaison if required. Follow-up was continued until the patient was clear of warts. Residual warts were treated with topical application of podophyllin or frozen with liquid nitrogen. Warts were considered to have recurred in any patient who during follow-up was cleared of anogenital warts and then subsequently developed new lesions.

The results of this treatment were assessed from the case notes with special reference to duration of symptoms, complications, postoperative analgesia, length of inpatient stay, and recurrence rates.

Results

Thirty-four patients (24 men and 10 women) were treated; 28 had been referred from the department of genitourinary medicine and six direct from the surgical unit. The mean age of the patients was 26·9 years (range 18-59 years). The mean duration of the warts was five months (range two weeks to three years). During this time various treatments, including topical application of podophyllin, freezing with liquid nitrogen, or electrocautery, had been used in all except eight patients. In the 24 men (with the exception of one patient with an extensive ridge of warts in the genitocrural fold) all warts were perianal; in eight there was intra-anal extension of the condylomata. One of these eight patients had a

Address for reprints: Dr K Slatford, Department of Genitourinary Medicine, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW

Accepted for publication 19 July 1982

corona of warts above the dentate line, which was treated by excision of a complete ring of mucosa and resutured as described above.

Of the 10 women, four had perianal warts alone and six perianal, vulval, introital, and intravaginal warts. In all the patients the extent of the lesions ranged from a moderate number of warts which had ceased to regress with conservative treatment to extensive circumanal vegetations causing considerable discomfort.

The only surgical complication was bleeding from an area of excision in one patient, which was easily controlled by a small under-running suture. Analgesia was not required in 17 patients while 17 were given a narcotic analgesic postoperatively.

The mean length of inpatient care was three days (range 2-8 days). Only six patients were in hospital for five days or longer, including one patient who bled postoperatively, one with extensive warts in the genitocrural fold, and one who stayed for social reasons. The remaining three patients had extensive intra-anal warts; as there is a risk of secondary haemorrhage in these patients they were kept in hospital for routine observation.

As six patients defaulted from follow-up complete data were available on only 28 cases. The mean length of follow-up was six weeks (range 4-16 weeks). During this time the mean number of attendances was three (range 1-8). Eight patients required no further treatment and two required only application of podophyllin at one attendance. These 20 patients were classed as primary cures—a rate of 71·4%. Of the remaining eight patients, cure was achieved within 16 weeks. In this group with a long follow-up some minor recurrences may have developed during treatment but this was difficult to assess. There were, however, three definite recurrences; one patient with a recurrence of circumanal warts required a further operation 12 months after his initial treatment and two developed recurrences four and six months after surgery but both were minor and responded readily to application of podophyllin. Allowing for the long follow-up group the recurrence rate in this series was 9·3%.

Discussion

The problem of tissue destruction exists with all direct treatments of anogenital warts. Whether treatment is chemical, by electrocautery, or by cryotherapy some normal surrounding tissue will be destroyed however accurate the application. This adverse effect is likely to increase discomfort and delay healing. In extensive warts it will also lead to excessive scarring and anal deformity. The method of scissor excision is unique in that it does not destroy surrounding tissue; only the abnormal tissue is removed and there is little damage to the normal tissue.[3]

The results in this series are comparable with the original series—a primary cure rate of 71·4% compared with 58% and a recurrence rate of 9·3% compared with 16%. The better results in our series may be accounted for by the smaller number of patients rather than a more skilful technique. The other result which can be compared is the use of postoperative analgesia. In our series 50% of cases required no sedation, which is similar to that in the original series.

This study has also highlighted some new points about this method. In addition to us wound inspections were also carried out by other medical and nursing staff of the genitourinary medicine department. All were impressed by the cosmetic result, evident by the complete lack of scarring, compared with other methods. The nursing staff were also impressed by the minimal discomfort to the patient during the application of dressings. We realise that these are subjective observations.

The use of this technique for treating genital warts in female patients was not mentioned in the original report. Although our numbers were small the technique would seem equally effective for this condition.

The mean length of stay in hospital was three days; this could have easily been shortened. Local arrangements made it convenient to admit the patient to hospital one day before the operation. As most of the patients were young and fit, however, they could have been admitted and fasted on the day of surgery, thus reducing the stay in hospital by one day. This would have reduced the length of stay to two days in most patients and to day-case admission in selected patients.

In conclusion, scissor excision appeared to be an excellent technique for treating anogenital warts in patients attending a department of genitourinary medicine, and our results compared well with those of the original series.

We are grateful to the staff of the Department of Genitourinary Medicine for their cooperation and help. J M Gollock thanks James P S Thomson for his instruction in the technique of scissor excision.

References

1. Abcarian H, Smith D, Sharon N. The immunotherapy of anal condyloma acuminatum. *Dis Col Rect* 1976;19:237-44.
2. Gosh AK. Cryosurgery of genital warts in cases in which podophyllin treatment failed or was contraindicated. *Br J Vener Dis* 1977;53:49-53.
3. Thompson JPS, Grace RH. The treatment of perianal and anal condylomata acuminata. *J R Soc Med* 1978;71:180-5.
4. Thomson, JPS. Perianal and anal condylomata acuminata. In: Rob C, Smith R, eds (1977), *Operative Surgery;* Todd IP, ed, *Colon, Rectum and Anus*. London: Butterworths, 1978:376-8.

Br J Vener Dis 1982;**58**:402-4

Screening for syphilis among homosexual men in bars and saunas in Amsterdam

L H LUMEY, JEANNETTE KOK, AND R A COUTINHO
From the Department of Infectious Diseases, Municipal Health Service, Amsterdam, The Netherlands

SUMMARY Serological screening of 1627 homosexual men visiting two saunas and two bars in Amsterdam showed that 32·2% were seroreactive for syphilis. The 18-month study period ended in June 1981. Follow-up was possible in 91% (224/245) of subjects with both positive TPHA and VDRL test results; 76 cases of previously undetected syphilis were identified among 75 of the 224 men (incidence rate 4·6%). The rate of previously undetected syphilis declined appreciably during the study period. The cost of identifying one new case of syphilis was Dfl577 (£120 or US$220).

Introduction

That some groups of homosexual men have much higher rates of syphilis than heterosexual men is well established.[1-3] Sexually active homosexual men visiting saunas seem to be especially at risk.[4-8] For this reason a pilot study was conducted among 318 homosexual men visiting two saunas in Amsterdam in August 1979, in which serological evidence of previous infection with *Treponema pallidum* was found in 34%.[9]

We decided to continue screening for syphilis in two bars and two saunas in Amsterdam to detect cases of untreated syphilis. Visitors to these locations were selected as contact tracing indicated they were a major source of syphilis. Cases of syphilis were referred to different physicians for further treatment.

Subjects and methods

STUDY POPULATION

The study population was as described in the pilot study.[9] In the present study blood samples were collected by venepuncture from male homosexuals in two saunas and two bars in Amsterdam, which were only accessible to men, between November 1979 and June 1981.

SEROLOGICAL TESTS FOR SYPHILIS

Sera were inactivated at 56°C for 30 minutes before testing. The serological tests used were the Venereal Disease Research Laboratory (VDRL) slide test (Wellcome) and the *Treponema pallidum* haemagglutination assay (TPHA, Fujizoki).

FOLLOW-UP AND STAGING

All patients with serological findings suggesting recent syphilis (VDRL and TPHA test results positive) were contacted by letter. We asked these patients' permission to discuss their diagnosis and subsequent treatment with the physicians they contacted for treatment; permission was generally granted when the aim of the follow-up study was explained. Patients not responding to our letter were contacted by telephone.

The clinical stage of the disease could be assessed since additional information from the treating physicians was available on clinical follow up and by repeat laboratory tests. Over 85% of patients had been treated by specialists in dermatovenereology.

STATISTICAL METHODS

The test against trend in a 2×k table was applied.

Results

A total of 1627 men participated in the study. Nearly half of them were between 25 and 35 years old (range 17-68 years). Of the men studied, 787 (48·2%) lived in Amsterdam, 683 (42·0%) elsewhere in the Netherlands, and 159 (9·8%) in 14 other countries. Participants were almost exclusively white (over 98%) and were living in Western Europe or the United States of America.

A total of 2385 blood samples was taken for serological testing at 52 screening sessions averaging six hours each. The mean number taken per session was 46 (range 15-80 samples) (table I).

Address for reprints: Dr R A Coutinho, Municipal Health Service, PO Box 20244, 1000 HE Amsterdam, The Netherlands

Accepted for publication 19 July 1982

TABLE I *Number of screening sessions, blood samples, and cases of previously undetected syphilis in male homosexual visitors to two saunas and two bars in Amsterdam*

Period of screening	*No of screening sessions*	*No of blood samples*	*Samples/session*	*Cases of untreated syphilis detected* (% of samples taken)*
1979 October—December	3	142	47·3	3 (2·1)
1980 January—March	8	374	46·8	25 (6·7)
1980 April—June	8	378	47·3	9 (2·4)
1980 July—September	10	461	46·1	16 (3·5)
1980 October—December	9	427	47·4	16 (3·7)
1981 January—March	7	332	47·4	5 (1·5)
1981 April—June	7	271	38·7	2 (0·7)
Total	52	2385	45·8	76 (3·2)

*Test against trend in a 2×k table: $t=-3 \cdot 15$; $P=0 \cdot 0008$.

SEROLOGICAL FINDINGS

The results of the serological findings in the men participating in the study are summarised in table II. In one-third (524/1627) of the men a positive TPHA result was found; nearly half of these cases (245/524) also had a positive VDRL slide test result. Follow-up was possible in 91% (224/245) of subjects with both positive TPHA and VDRL test results; 75 of the 224 men were treated for previously undetected syphilis after serological screening. Over 85% (64/75) of patients had been treated by specialists in dermatovenereology in the Netherlands or at the clinics for sexually transmitted diseases (STDs) of the Municipal Health Service in Amsterdam. Six patients (8%) had been treated in other countries. The remainder (five patients) had received treatment from family practitioners. One patient was infected twice during the study period. There was no difference in the findings at the different locations.

The clinical stages of syphilis of the patients was as follows: primary syphilis, nine cases; secondary syphilis, seven; early latent syphilis, 24; late latent, 10; and unknown, 26.

DECREASING INCIDENCE IN STUDY PERIOD

The test against trend in a 2×k table was applied to the figures in table I. This test relates the number of cases of untreated syphilis detected to the number of blood samples taken. This test gave a $t=-3 \cdot 15$ which, as it is a one-sided test here, gave $P=0 \cdot 0008$. This means that rates of previously undetected syphilis (new cases/blood samples taken) declined significantly during the study period.

COSTS

The cost of staff-hours spent taking blood samples and performing laboratory investigations amounted together to Dfl 44 000. Thus, the cost of identifying one patient with previously undetected syphilis in our study was Dfl 577 (about £120 or US$220).

Discussion

Incidence rates of 1-3% have been reported[4-8] in screening programmes for syphilis among male visitors to gay baths in the United States of America. In this study the incidence of syphilis was 4·6%

TABLE II *Serological findings by age in homosexual visitors to two saunas and bars between November 1979 and June 1981. (Numbers in brackets indicate men who gave histories of treated syphilis.)*

	Test results:						
	VDRL− TPHA−	*VDRL− TPHA dubious*	*VDRL− TPHA+*	*VDRL+ TPHA+*			
Age group (years)				*Adequately treated*	*No follow-up*	*Untreated*	*Total*
≮24	150 (3)	2 (2)	17 (13)	9 (9)	4 (1)	6 (2)	188 (30)
25-34	509 (25)	19 (12)	142 (108)	64 (64)	5 (2)	44 (19)	783 (230)
35-44	275 (25)	18 (11)	95 (78)	56 (56)	10 (3)	20 (11)	474 (184)
≯45	123 (8)	7 (5)	25 (16)	20 (20)	2 (2)	5 (2)	182 (53)
All ages	1057 (61)	46 (30)	279 (215)	149 (149)	21 (8)	75* (34)	1627 (497)
Percentage of total	65	2·8	17·1	9·2	1·3	4·6	100

*75 patients, 76 cases

(75/1627). We do not think that some cases could have had yaws (framboesia) because only two of the 75 patients were non-whites from Surinam. One case seroconverted from negative to highly positive, and in another (who gave a history of syphilis) a fourfold increase in VDRL titres was found before treatment. Both cases were classified as latent syphilis.

One-third (524/1627) of the participants of the study had a positive TPHA test result. This was the only positive serological finding in 279 men, of whom 215 volunteered a history of syphilis. The TPHA has been evaluated in a number of studies and is highly specific and sensitive for the detection of treated and untreated cases of syphilis.[10-13] We, therefore assumed that the isolated positive TPHA results in sera of 279 patients were not biological false-positive reactions. Thus, 32·2% (524/1627) of the participants in the study were seroreactive for syphilis. This is in accordance with the findings of our pilot study.[9]

The VDRL slide test has been evaluated by Notowicz[14] among others. His evaluation suggested that the predictive value of a positive VDRL slide test for the detection of untreated cases of syphilis was much higher than the predictive value of a positive TPHA result. We therefore regarded patients with a negative VDRL slide test result as having been adequately treated. These patients were therefore not included in our follow-up.

The follow-up of 91% (224/245) of subjects with both positive VDRL and TPHA test results was possible because of the facilities for diagnosis, treatment, and contact-tracing of STD that exist in the Netherlands. Serological diagnosis of syphilis is provided free of charge by regional public health laboratories and treatment is provided free of charge by STD clinics in the major cities and by private specialists in smaller towns. The costs are borne by the National Ministry of Health, which also employs the contact-tracing staff.

In this study screening for syphilis in bars and saunas coincided with a significant fall in rates of previously undetected syphilis. A causal relationship between screening and the declining rates seems plausible but cannot be inferred on the basis of our findings alone.

The cost of identifying one patient with untreated syphilis in our study (£120 or US$220) was low compared with the costs of traditional methods of finding cases in STD clinics.

In this study simple serological screening in high-risk locations in Amsterdam has been effective in detecting untreated cases of syphilis. We decided therefore to continue the programme. As the rates of undetected syphilis, however, declined during the programme the interval between the screening sessions was increased. If the rate starts rising again, we shall adjust the interval accordingly.

Blood samples were taken by Nelleke van den Akker, Lidwien Berkhout, Thérèse van der Helm, Fem Jansen Schoonhoven, Jeannette Kok, and Liesbeth Lanser (Municipal Health Service, Amsterdam). Serological testing was carried out at the bacteriological laboratory of the Municipal Health Service, Amsterdam. We thank Dr Hilda Kuipers for her statistical advice.

References

1. Fluker JL. A 10-year study of homosexually transmitted infection. *Br J Vener Dis* 1976;**52**:155-60.
2. Thin RN. Some characteristics of homosexual men. *Br J Vener Dis* 1976;**52**:161-4.
3. British Co-operative Clinical Group. Homosexuality and venereal disease in the United Kingdom. *Br J Vener Dis* 1980;**56**:6-11.
4. Ritchey MG, Leff AM. Venereal disease control among homosexuals. An outreach program. *JAMA* 1975;**232**:509-10.
5. Judson FN, Miller KG, Schaffnit TR. Screening for gonorrhea and syphilis in the gay baths – Denver, Colorado. *Am J Public Health* 1977;**67**:740-2.
6. Merino HI, Richards JB. An innovative program of venereal disease case-finding, treatment and education for a population of gay men. *Sex Transm Dis* 1977;**4**:50-2.
7. Merino HI, Judson FN, Bennett D, Schaffnit ThR. Screening for gonorrhea and syphilis in gay bathhouses in Denver and Los Angeles. *Public Health Rep* 1979;**94**:376-9.
8. Wolf FC, Judson FN. Intensive screening for gonorrhoea, syphilis and hepatitis B in a gay bath-house does not lower the prevalence of infection. *Sex Transm Dis* 1980;**7**:49-52.
9. Bleeker A, Coutinho RA, Bakker-Kok J, Tio D, de Koning GAJ. Prevalence of syphilis and hepatitis B among homosexual men in two saunas in Amsterdam. *Br J Vener Dis* 1981;**57**:196-9.
10. Garner MF, Backhouse JF, Daskalopoulus W, Walsh JL. *Treponema pallidum* haemagglutination test for syphilis: comparison with the TPI and FTA-ABS tests. *Br J Vener Dis* 1972;**48**:470-2.
11. Young H, Henricksen C, Robertson DHH. *Treponema pallidum* haemagglutination test as a screening procedure for the diagnosis of syphilis. *Br J Vener Dis* 1974;**50**:341-6.
12. Luger A, Schmidt B, Spendlingwimmer I, Horn F. Recent observations on the serology of syphilis. *Br J Vener Dis* 1980:**56**:12-6.
13. Luger A, Schmidt BL, Spendlingwimmer I, Steyrer K. Specificity of the *Treponema pallidum* haemagglutination test. Analysis of results. *Br J Vener Dis* 1981;**57**:178-80.
14. Notowicz A. Serological tests in venereal syphilis. *PhD thesis*, Rotterdam: Erasmus Universiteit Rotterdam, 1981.

Br J Vener Dis 1982;**58**:405-7

Ulcerative vulvitis in Reiter's syndrome

A case report

S O'N DAUNT, K E KOTOWSKI, A P O'REILLY, AND A T RICHARDSON
From the Royal Free Hospital, London

SUMMARY In a case of acute Reiter's syndrome with severe vulvitis the diagnosis was based on the presence of a vaginal discharge and dysuria, arthritis, conjunctivitis, buccal ulceration, keratodermia blenorrhagica, and HLA B27 tissue-typing antigen. The vulval lesions were similar in appearance to those of circinate vulvitis. The acute histological change were confined to shallow ulceration with an inflammatory infiltration of the subjacent dermis. Coincidential lichen sclerosus et atrophicus was present, which could have been masked by the acute lesions.

Introduction

Reiter's syndrome is uncommon in women,[1] and vulval lesions have been reported previously in only three cases.[2 3] In two of these cases shallow circinate erosions were the major feature.[3] Our patient had similar lesions, and we report for the first time the histological changes as well as the clinical course.

Case report

A 28-year-old unmarried Caucasian woman presented with an acute asymmetrical inflammatory arthropathy affecting the right knee, both ankles, and the right shoulder. One month before its onset she had experienced explosive watery diarrhoea lasting three days. This was followed three weeks later by a vaginal discharge, dysuria, frequency of micturition, mild conjunctivitis, and painless buccal ulceration. She denied any previous urogenital symptoms.

CLINICAL FEATURES

She was febrile (temperature 39·7°C) and effusions were present in the right knee and ankle. There was a pronounced vulvitis consisting of a widespread area of erythema around the introitus and extending to the perianal region. The vulva was covered by many scattered, well-defined, round shallow ulcers, and an underlying atrophy was suspected. A thick white vaginal discharge was present, but the cervix appeared healthy. There was no urethral discharge. Based on these appearances, and supported by a consultant dermatologist's opinion, a combined diagnosis of circinate vulvitis and suspected lichen sclerosus et atrophicus was made. There were no other skin lesions, and general examination including sigmoidoscopy was otherwise normal.

INVESTIGATIONS

Microscopy of vaginal smears and culture indicated the presence of candidosis. Urethral, vaginal, cervical, and rectal cultures for *Neisseria gonorrhoeae, Trichomonas vaginalis,* and *Chlamydia trachomatis* gave negative results. Stool cultures for *Salmonella* spp, *Shigella* spp, *Yersinia* spp, and *Campylobacter* spp also gave negative results. *Klebsiella oxytoca* was present in the first stool specimen but not on three following occasions. A mid-stream urine specimen contained $0 \cdot 4 \times 10^9/1$ white blood cells, but culture for eubacteria gave a negative result. Synovial fluid aspirated from the right knee contained $9 \cdot 4 \times 10^9/1$ white blood cells (90% mature polymorphonuclear cells and 10% small mononuclear cells with active chromatin), but culture for bacteria, including gonococci, gave negative results.

Paired sera for Widal testing, complement fixation tests for psittacosis, mycoplasmas, and herpes simplex virus, and microimmunofluorescence for chlamydia all gave negative results.

The haemoglobin concentration was 10·3 g/dl, white cell count $7 \cdot 8 \times 10^9/1$ and erythrocyte sedimentation rate (Westergren) 120 mm in the first hour. Tissue typing showed that HLA B27 was present.

Fluorescent antibody tests for antinuclear factor and antibodies to mitochondria, gastric parietal cells,

Address for reprints: Dr S O'N Daunt, Rheumatology Department, Royal Free Hospital, Pond Street, London NW3 2QG

Accepted for publication 19 July 1982

thyroid colloid, and thyroid epithelium gave negative results. Rheumatoid factor was absent. Protein electrophoresis showed an acute-phase reaction with raised alpha$_2$ globulin and lowered albumin. The Venereal Diseases Research Laboratory (VDRL) and *Treponema pallidum* haemagglutination (TPHA) tests gave negative results.

X-ray films of chest, knees, ankles, and feet showed no abnormalities, as did a rectal biopsy. Vulval biopsy showed changes consistent with lichen sclerosus et atrophicus (fig 1). Additional focal areas of ulceration were present with an inflammatory infiltration of the subjacent dermis (fig 2).

The patient's regular boyfriend (and only recent sexual contact) was examined. Neither *N gonorrhoeae* nor *C trachomatis* was isolated from the urethra.

TREATMENT AND COURSE OF ILLNESS

The patient was initially treated with rest in bed and given piroxicam, naproxen, and intra-articular steroid injections. Her fever subsided after 48 hours, and the arthritis gradually disappeared over the next six weeks. On the advice of a consultant venereologist a two-week course of erythromycin and nystatin pessaries was given once all the specimens for culture had been collected. The vulval lesions, which were treated with salt baths and local miconazole nitrate 2% and hydrocortisone 1% cream, had healed two months later.

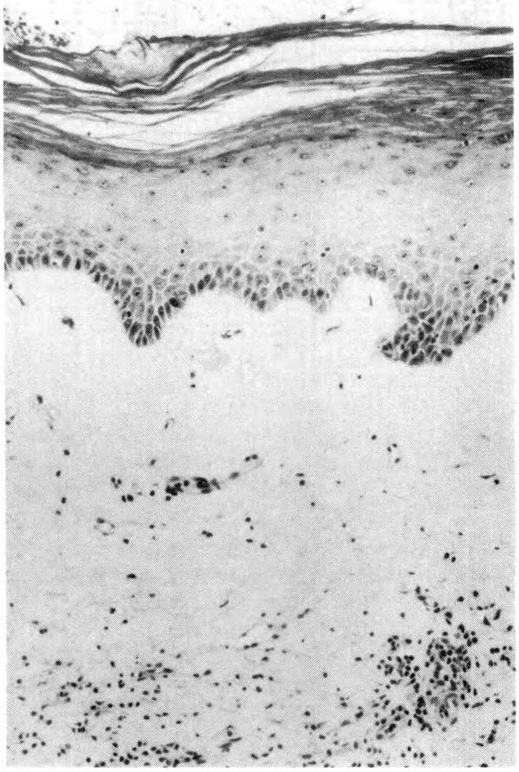


FIG 1 *Vulval biopsy showing hyperkeratotic, non-ulcerated epidermis and papillary dermis composed of oedematous homogenised connective tissue consistent with lichen sclerosus et atrophicus.* × *100.*

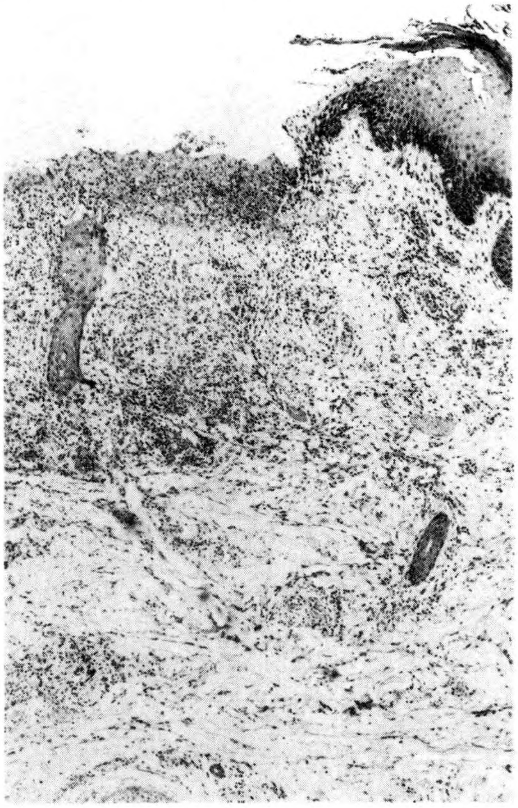


FIG 2 *Vulval biopsy showing focal ulceration of the epidermis with acute inflammation of the immediately subjacent dermis.* × *40.*

Two weeks after presentation she developed a few brown papules on the sole of the left foot, the appearance being consistent with keratodermia blenorrhagica; these disappeared spontaneously three weeks later.

Three months after her first presentation all symptoms and signs of Reiter's syndrome had resolved; the vulval atrophy did not progress. The erythrocyte sedimentation rate fell to 20 mm in the first hour and the haemoglobin concentration rose to 12·0 g/dl. All treatment was stopped. At five months she had developed atrophic lesions of the skin, which were typical of lichen sclerosus et atrophicus, over the left shoulder and reticulate lesions on the buccal mucosa. She refused biopsy of these lesions. At one year the lesions had progressed no further, and there has been no recurrence of the Reiter's syndrome.

Discussion

This woman's presentation was typical of post-dysenteric Reiter's syndrome. She had at least five of the features of the clinical hexad[4] (when this is modified for her sex)—namely arthritis, conjunctivitis, mucosal lesions, keratodermia blenorrhagica, and circinate vulvitis. The diagnosis was also supported by the presence of HLA B27. Although urethritis was not proved, a history of dysuria and white cells in the urine does suggest that it may have been present. Urethritis is not, however, essential to the diagnosis of Reiter's syndrome, as Paronen's[5] large study found it to be absent in 23% of cases; indeed cystitis has been proposed as the female counterpart for urethritis.[6]

The candidosis was probably coincidental, as in one of the cases reported by Thambar.[3] Unfortunately, we were unable to determine the precipitating organism for the Reiter's syndrome in this case.

The histological changes of ulceration are the first to be recorded. No other acute features were present; in particular no psoriatic changes could be found, but this has to be confirmed by future cases. A biopsy is indicated because underlying disease, which could otherwise be masked, may be missed.

The lichen sclerosus et atrophicus was probably a coincidental but important finding. An increased incidence of organ-specific antibodies (absent in this case) has been noted in this condition,[7 8] and vulval lesions may carry an increased risk of neoplastic change.[9] Follow-up of such patients is advised[10]; it might have been overlooked in this case if the lichen sclerosus et atrophicus had not been confirmed by the biopsy.

We wish to thank the illustration section of the department of pathology, Royal Free Hospital, and Miss D M Green for their assistance in the preparation of the manuscript.

References

1. Boyle JA, Watson Buchanan W. *Reiter's Disease; Clinical Rheumatology,* Oxford and Edinburgh: Blackwell Scientific Publications, 1971.
2. Reich H. Lymphknoten beteiligung bei Reitercher Kranheit (Reiter-lymphadenitis). *Hautarzt* 1966;17;406-9.
3. Thambar IV, Dunlop R, Thin RN, Huskisson EC. Circinate vulvitis in Reiter's syndrome, *Br J Vener Dis* 1977; 53; 260-2.
4. Katz WA. *Reiter's Disease, Rheumatic Disease: Diagnosis and Management.* Philadelphia: J B Lippincott & Co, 1977.
5. Paronen I. Reiter's disease—a study of 344 cases observed in Finland. *Acta Med Scand* 1948;131 suppl:212.
6. Hollander JL, McCarty DJ. *Reiter's Syndrome, Arthritis and Allied Conditions,* 8th ed. Philadelphia: Lea and Febiger, 1972.
7. Harrington CI, Dunsmore IR. An investigation into the incidence of auto-immune disorders in patients with lichen sclerosus et atrophicus, *Br J Dermatol* 1981;104;583-89.
8. Goolamali SK, Barnes EW, Irvine WJ, Shuster S. Organ specific antibodies in patients with lichen sclerosus. *Br Med J* 1974;iv:78-9.
9. Wallace HJ. Lichen sclerosus et atrophicus, *Transactions of St John's Dermatological Society,* 1971;57:9-30.
10. Ridley CM. *Dermatoses of the Vulva: Major Problems in Dermatology,* vol 5, London: W B Saunders & Co Ltd, 1975.

Br J Vener Dis 1982;**58**:408-9

Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Penicillinase-producing *Neisseria gonorrhoea* in Jakarta, Indonesia

Sir,
Between 30 April 1981 and 30 April 1982, 156 cases of gonorrhoea were confirmed by culture in the department of microbiology of the Medical Faculty, University of Indonesia. Of these cases, 39 were due to infection with penicillinase-producing *Neisseria gonorrhoeae* (PPNG). No PPNG strains had been detected in this department before April 1981. All 156 cases occurred in Indonesian patients, both male and female. The first impression was that men seemed to be more affected by PPNG infections than women (table I). Statistically the difference was not significant at the 1% level ($\chi^2=0{\cdot}42$, $P<0{\cdot}5$). Does this imply that men and women run a similar risk of developing PPNG infections?

Isolation and identification of PPNG and non-PPNG strains were carried out according to standard methods. The minimum inhibitory concentration of penicillin was measured by a plate dilution method. All PPNG strains showed resistance to 128 μg/ml penicillin G. Further testing of higher concentrations of penicillin could not be performed since these 39 PPNG strains did not remain viable. Several PPNG strains collected later (after April 1982) were tested at higher concentrations of penicillin (table II); none of the 14 PPNG strains showed any inhibition of growth on 10-unit penicillin discs.

Yours faithfully,
S Josodiwondo

Department of Microbiology,
Medical Faculty, University of Indonesia,
Pegangsaan Timur 16,
Jakarta, Indonesia.

TABLE I *Number of PPNG infections diagnosed between 30 April 1981 and 30 April 1982*

Patients' sex	*PPNG strains*	*Non-PPNG strains*	*Total No of cases*
Male	37	105	142
Female	2	12	14
Total	39	117	156

TABLE II *Minimum inhibitory concentrations (MICs) of penicillin for 14 PPNG strains*

	MIC of penicillin (μg/ml):				
	128	*256*	*1024*	*2048*	*4096*
PPNG Strains	3	1	1	1	8

TO THE EDITOR, *British Journal of Venereal Diseases*

Changes in intravascular kininogen during the Jarisch-Herxheimer reaction in secondary syphilis

Sir,
The pathogenesis of the Jarisch-Herxheimer reaction (JHR) after the treatment of syphilis remains obscure. It has been attributed to the release of treponemal breakdown products and release of endogenous leucocyte pyrogen[1] after the administration of treponemicidal drugs. Loveday[2] and others[3] have reported falls in early components of the classical pathway of the complement system which paralleled the severity of the preceding JHR. These workers proposed a scheme for the mode of activation of complement, kinin formation, and other inflammatory mediators and their possible relation to the clinical features of the JHR. We report changes in intravascular kininogen in six patients with early secondary syphilis and in one control subject in the first eight hours after the initial therapeutic dose of antibiotics.

Six seropositive patients (aged 26-66 years) with secondary syphilis who had a typical rash and minimal or no lymphadenopathy were studied. After being given 600 000 units of procaine penicillin (Distaquine suspension) by intramuscular injection they were observed for at least eight hours. Before treatment pulse, blood pressure, temperature, and clinical features were recorded and blood samples taken. Treatment was given and observations repeated and specimens taken at ½, 1, 1½, 2, 3, 4, 6, and 8 hours. Each blood sample was heparinised (100 U heparin/10 ml blood) avoiding contact with glass, and the plasma was separated within 30 minutes and frozen at −70°C.

Assay of plasma kininogen was expressed as bradykinin in μmol/l released from plasma kininogen by trypsin. The kinin released was assayed on the isolated rat uterus.[4] Immunoglobulin assays for IgG, IgM, and IgA were carried out using standard Hyland Immunoplates; results were expressed as percentages of those pooled for normal human serum.

The six patients showed varying degrees of clinical change after the start of treatment. In case 1 the patient had a severe JHR; the rash increased in intensity and the temperature rose to 38·8°C and the pulse rate to 100/minute; a slight fall in pulse rate produced a subsequent rise in blood pressure. This patient developed rigors, and observations and collection of blood samples were stopped at the six-hour interval. In case 2 the patient had a similar reaction with an increase in the intensity of the rash, a rise in temperature to 38·8°C, an increase in pulse rate to 110/minute, and a similar but more pronounced change in blood pressure than in case 1. Both these patients reported severe symptoms including myalgia, arthralgia, anorexia, and nausea. In cases 3 and 4 the patients had less severe reactions with rises in temperature to 38·9°C and 38°C respectively and an increase in pulse rate to 100/minute. Both patients showed a slight rash potentiation and felt nauseated and unwell for short periods. In cases 5 and 6 and the control subject 7 no reactions either objective or subjective occurred. When they did occur reactions began three to four hours after initiation of treatment.

A fall in intravascular kininogen occurred in several patients. This fall was most pronounced in the patients in cases 1 and 2 who had severe JHRs (figure). In case 1 the patient had a fall in intravascular kininogen of 60% (from 5·2 to 2·1 μmol equivalents of bradykinin/litre of plasma), the maximum occurring between the 1½- and three-hour intervals; in case 2 the patient had a fall of 96% (from 2·85 to 0·18 μmol equivalents of bradykinin/litre of plasma), the maximum occurring between the one- and three-hour intervals. In both patients values returned to normal by the six-hour interval. In cases 3 and 4 the patients had a less pronounced fall in kininogen of 16% and 23% respectively, which was shorter in duration. No fall in intravascular kininogen occurred in cases 5 and 6 or in the control subject 7. The intravascular concentrations of IgG, IgM, and IgA rose slightly over the first four hours in cases 1 and 2; in cases 3 and 4 and the control subject 7 no overall changes occurred during the period of observation.

The JHR in the patients studied was associated with an early reduction in intravascular kininogen values, reflecting pronounced kinin formation. The degree of this change in individual patients paralleled the severity of the reaction and we propose that kinin may play a part in the pathogenesis of the JHR. It is unlikely that falls in kininogen values are due to non-specific extravasation of plasma proteins or transient haemodilution, since during this time the concentrations of three major classes of immunoglobulins tended to rise or remain steady.

Depletion of prekallikerein, an intermediate enzyme precursor in the plasma kinin forming system, and other protein mediators in *Borellia recurrentis* infection has been shown during the JHR two hours after treatment; these values returned to normal in the convalescent period.[5] Other workers[6] failed to show plasma kinin formation during the JHR in secondary syphilis but they omitted to take blood samples before the three-hour interval.

The scheme proposed by Loveday[2] and others[3] suggested several possible sites of kinin formation during the JHR: via immune complexes, Hageman factor, early complement components, and lysozymal enzymes. Kinins so formed would have biological properties to mediate, at least in part, the subsequent reaction. In addition they proposed that the main site of the JHR in syphilis was extravascular. This may explain why only the patients having severe reactions (and pronounced activation of kininogen) showed significant changes in kininogen in the intravascular compartment. The rapid return to normal values after 3-4 hours may not reflect cessation of activation but merely an increase in overall synthesis.

Further work is in progress to clarify the role of plasma enzyme systems in the pathogenesis of the JHR.

Clive Loveday
J S Bingham

Department of Genitourinary Medicine,
James Pringle House,
Middlesex Hospital,
London W1N 8AA

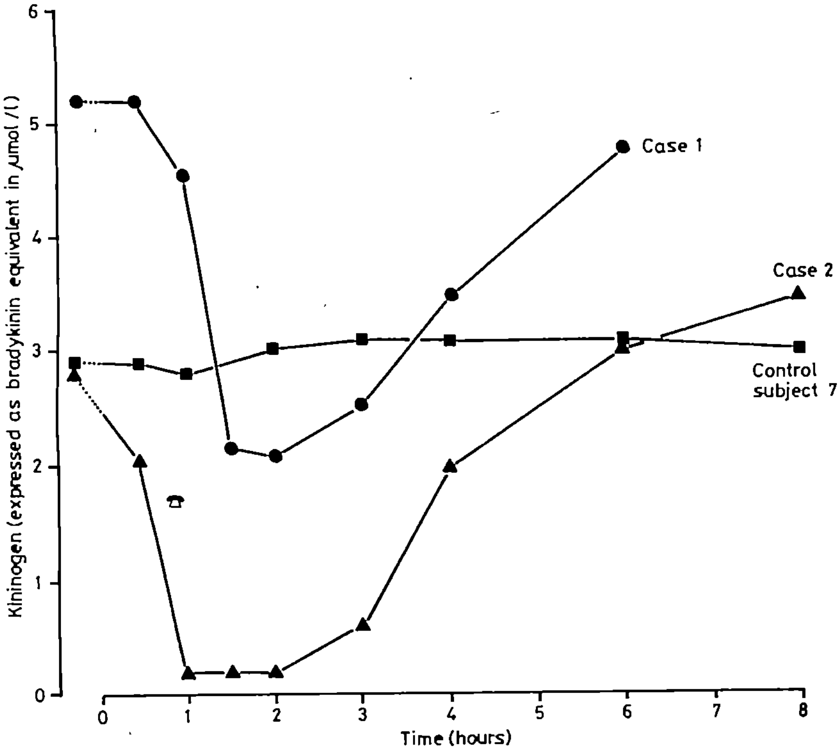


FIGURE Kininogen changes in cases 1 (●) and 2 (▲) and in the control subject 7 (■). In the two patients who had severe JHR maximum falls in intravascular kininogen occurred at one and two hours after treatment. No change was observed in the control subject.

References

1. Bryceson ADM. Clinical pathology of the Jarisch-Herxheimer reaction. *J Infect Dis* 1976;133:696-704.
2. Loveday C. Blood changes in inflamation and related processes. *PhD Thesis*. London: Council for Academic Awards, 1974.
3. Fulford KWM, Johnson N, Loveday C, Storey J, Tedder RS. Changes in intravascular complement and anti-treponemal antibody titres preceeding the Jarisch-Herxheimer reaction in secondary syphilis. *Clin Exp Immunol* 1976;24:483-91.
4. Eisen V, Loveday C. In-vivo effects of cellulose sulphate on plasma kininogen, complement, and inflamation. *Br J Pharmacol* 1971;42:383-91.
5. Galloway RE, Levin J, Butler T, *et al*, Activation of protein mediators of inflamation and evidence for endotoxemia in *Borrelia recurrentis* infection. *Am J Med* 1977;63:933-8.
6. Zachariae H, Neilsen E. Plasma kinins and the Jarisch-Herxheimer reaction. *Acta Dermatovenereol* 1974;54:401-2.

Br J Vener Dis 1982;**58**:410

Book reviews

Sexually Transmitted Diseases and VD. By R D Catterall, 1982. Family Doctor Publications, British Medical Association, London. Pp 30. Price: £0.50.

This booklet, produced by the British Medical Association, is certainly a most complete guide to the sexually transmitted diseases (STD). Indeed it would be useful reading for medical students and even medical practitioners working with young people. It is, however, going to be largely wasted on the group most at risk from these diseases, namely adolescents and young adults.

The general format is drab with a tendency for unnecessary repetition. Infections are dealt with not only individually but also as they might present. A problem-orientated approach, which stresses the possibility of asymptomatic infection, is probably more helpful to the teenager reading such a booklet. The section on signs and symptoms should therefore have been expanded with more detailed information on the specific infections described later.

Although well written the language used is extremely complex with a Havering Index of over 15. This equates with the reading age of a bright grammar school pupil and would deter the average reader from completing the booklet, which is unfortunate as sections at the end entitled "The STD clinics," "Questions you ask," and "Ten points you should know" are useful. The non-medical reader will be confused by the use of technical terms without sufficient explanation, such as "intra-uterine device," "septicaemia," "penicillin sensitivity," and "catheter." A note of the colloquial terms for the venereal infections, parts of the anatomy, and symptoms would have been more valuable.

A text aimed at health education should be accompanied by clear simple drawings and diagrams to illustrate the concepts described. The drawings in this booklet are of couples kissing, cuddling, drinking, or going to a clinic. They are irrelevant and therefore valueless. Simple drawings of the genitalia, showing how the fallopian tubes might become blocked or what a "sore" looks like, would have greatly enhanced the text.

In conclusion this booklet will be useful to a comparatively small group of school teachers and paramedics concerned in health education and possibly to intelligent worried parents. It will be of little value to the young at risk and regrettably cannot be recommended to the average clinic attender requesting further information.

I should like to gratefully acknowledge the help of Mr Ian Fairfax (Area Health Education Officer, Newcastle Health Authority) for his advice while preparing this review.

R S Pattman

Treponemal infections: Report of a WHO Scientific Group. WHO Technical Report Series No 674. 1982. WHO, Geneva (available at HMSO, London). Pp 75. Sw frs 9.

In 1978 the World Health Assembly requested the director general of the World Health Organisation to draw up, disseminate, and update as required guidelines on the control of sexually transmitted diseases. This excellent report was accordingly produced following a convention of the WHO Scientific Group on Treponemal Infections in 1980.

The book deals in a logical sequence with the epidemiology, clinical aspects, diagnosis, management, control, and research aspects of syphilis and the non-venereal treponematoses outlining changes within the past 10-20 years. An admirable balance is achieved between the management of syphilis in developed countries with their resources for diagnosis, follow-up, and contact tracing and that in the developing countries where these facilities are often not available. Clear and unequivocal recommendations are made throughout the book with particularly useful guidelines on the treatment of the various types and stages of treponemal infection. These are all concisely summarised at the end in a most practical fashion. The penultimate chapter provides a useful synopsis on research aspects, and the section entitled "New parameters for the diagnosis of neurosyphilis by cerebrospinal fluid examination" is especially valuable, meriting further investigation. The information on immunity, however, is scanty considering the amount of current research in this field.

This impressive volume should be read by all clinicians, laboratory workers, and public health administrators in any way concerned with treponemal infection. At the modest price of £2.10 there is no reason why it should not be readily available.

R S Pattman

Notice

In recognition of his lifetime contribution to the field of sexually transmitted diseases Dr R D Catterall has been awarded the Thomas A Parran award for 1982 from the American Venereal Diseases Association. It is particularly pleasing that a British physician who has accomplished much in the promotion of the specialty, both nationally and internationally, should have been presented with this prestigious award.

Br J Vener Dis 1982;**58**:411-7

Index to Volume 58, 1982

Authors and subjects are indexed together. There is a separate index of articles abstract and another of authors of articles abstracted.

AARDOOM H A, DE HOOP D, ISERIEF C O A, MICHEL M F, and STOLZ E: Detection of *Neisseria gonorrhoeae* antigen with a solid-phase enzyme immunoassay, **359**
Abortion, therapeutic, and *C trachomatis* infection, **182**
ACKERS J P. *See* SOLIMAN M A *et al,* **250**
ACKERS J P. *See* STREET D A *et al,* **330**
ADELUSI B. *See* DAROUGAR S *et al,* **366**
Adenovirus type 19 and a closely related new serotype in genital infections, **131**
ADLER M W, BELSEY E H, O'CONNOR B H: Morbidity associated with pelvic imflammatory disease, **151**
ADLER M W: Consulting patterns after a television programme on sexually transmitted diseases, **259**
AL-OMAR L S. *See* BARTON I G *et al,* **44**
AL-QUDAH A A and MOSTRATOS A: Reiter haemagglutination test: a screening test for syphilis, **281**
Amsterdam, syphilis screening among homosexuals in bars and saunas, **402**
Anaerobes in men with urethritis, **321**
ANDERSON P. *See* MILLER M A *et al,* **96**
ANDERSON R. *See* DE KLERK E *et al,* **275**
Ankylosing spondylitis occurring simultaneously with rheumatoid arthritis and Reiter's syndrome, **196**
ARAMESH B. *See* DAROUGAR S *et al,* **374**
ARKO R J, WONG K H, FINLEY-PRICE K G, RASHEED J K: Effects of tampon components on growth and dissemination of *Niesseria gonorrhoeae,* **105**
ARSENAKIS M, MAY J T: Confirmation of herpes simplex virus type 2 infections in herpes-like genital lesions by simple complement-fixation test, **48**
ARYA O P. *See* MALLINSON H *et al,* **36**
ATIA W A, RATNATUNGA C S, GREENFIELD C, and DAWSON S: Aseptic meningitis and herpes simplex proctitis: a case report, **52**
ATIA W. *See* RIDGWAY G L *et al,* **236**
AUSTIN T W, SMITH E A, DARWISH R, RALPH E D, and PATTISON F L M: Metronidazole in a single dose for the treatment of trichomoniasis: failure of a 1-g single dose, **121**
Australia and Sweden, comparison of social factors in homosexually acquired VD, **263**

BACKHOUSE J L. *See* NEY R *et al,* **351**
Balanitis, circinate, ultrahistopathology of, **188**
Balanoposthitis associated with *G vaginalis* infection, **127**
BALSDON M J, LAING S R S, and TAYLOR G E: Postgonococcal cervicitis and postgonococcal urethritis (correspondence), **275**
BARLOW D. *See* BLACKWELL A, **387**
BARRISON I G. *See* VIOLA L *et al,* **59**
BARTON I G, KINGHORN G R, NAJEM S, ALOMAR L S, POTTER C W: Incidence of HSV-1 and HSV-2 isolated from a group of patients with herpes genitalis in Sheffield, **44**
BELSEY E H: Epidemiological treatment of gonorrhoea and non-specific genital infection in female sexual contacts: current practices in STD clinics in England and Wales, **113**
BELSEY E H. *See* ADLER M W *et al,* **151**
BHAT G J. *See* RATNAM A V *et al,* **357**
BINGHAM J S. *See* LOVEDAY C, **468**
BLACKWELL A and BARLOW D: Clinic diagnosis of anaerobic vaginosis (non-specific vaginitis): a practical guide, **387**
BOEDISANTOSO M. *See* SOENDJOJO A *et al,* **149**
BOOK REVIEW
  CATTERALL R D: Sexually Transmitted Diseases and VD (1982), **410**
  COLLART P and POITEVIN M: Actualités Physiobiologiques sur la Syphilis: Monographie, Semaine des Hôpitaux (1981), **133**
  FARID M. Sexually Transmitted Diseases (1981), **205**
  NOBLE R C: Sexually Transmitted Diseases (1982), **343**
  ORIEL J D and RIDGWAY G L: Genital Infection by *Chlamydia trachomatis:* Current Topics in Infection (series 2) (1982), **342**
  THIN R N T: Lecture Notes on Sexually Transmitted Diseases (1982), **343**
  WHO: Treponemal Infections: Report of a WHO Scientific Group (1982), **410**
BREGUET D. *See* NEY R *et al,* **351**
BREGUET G. *See* NEY R *et al,* **351**
Brussels, in-vitro activity of 21 antibiotics against *N gonorrhoeae* in, **23**
BRUUN L. *See* FENNESTAD K L *et al,* **143**
BUCHAN A. *See* SKINNER G R B *et al,* **381**
BUTZLER J P. *See* GORDTS B *et al,* **23**

CAHN T. *See* FIUMARA N J, **202**
CALUBIRAN O V, CRISOLOGO-VIZCONDE L B, TUPASI T E, TORRES C A, and LIMSON B M: Treatment of uncomplicated gonorrhoea in women: comparison of rosoxacin and spectinomycin, **231**
CALUBIRAN O V. *See* TUPASI T E *et al,* **176**
Candidosis, vaginal, treatment with single 500 mg clotrimazole pessary, **124**
CATLIN B W, REYN A: *Neisseria gonorrhoeae* isolated from disseminated and localised infections in pre-penicillin era: auxotypes and antibacterial drug resistances, **158**
CATLIN B W. *See* EXNER A C *et al,* **166**
CATTERALL R D: International symposium on trichomoniasis: the Polish medical scene, **56**
CATTERALL R D: IUVDT—South-east Asian and Western Pacific regional conference, **273**
CATTERALL R D. Sexually transmitted diseases inIndia and Sri Lanka, **338**

CATTERALL R D: STD—Sixth National Conference of the Indian Association, **340**

CATTERALL R D: World and Latin American congress on sexually transmitted diseases, **204**

CATTERALL R D: *See* SOLIMAN M A *et al*, **250**

Cefaclor, single oral dose for penicillinase-producing *N gonorrhoeae*, **176**

Ceftriaxone for treatment of *N gonorrhoeae*, **314**

Cervicitis, postgonococcal and postgonococcal urethritis, **275**

Cervix, traumatic ulceration of, **202**

CHAN J K, SCHELL R F, and LE FROCK J L: Mitogenic responses of hamsters infected with *Treponema pertenue:* lack of correlation with passive transfer of resistance, **292**

Chancroid, treatment with thiamphenicol, **54**

Chlamydia trachomatis infection and therapeutic abortion, **182**

C trachomatis, quantitative study of, in genital infection, **36**

Chlamydial culture service to STD clinic, **236**

Chlamydial genital infection in Ethiopia, seroepidemiological survey of, **370**

Chlamydial genital infection in Nigeria, seroepidemiological survey of, **366**

Chlamydial screening of pregnant women in STD clinic, **327**

Chlamydial urethral infection in male STD clinic patients in Teheran, **374**

CHOWDHURY F H. *See* KINGHORN G R *et al*, **127**

Cimetidine, inefficacy in genital warts, **275**

CLAY J C. *See* PENN C W, **286**

CLAY J C. *See* SKINNER G R B *et al*, **381**

Clotrimazole, single 500-mg pessary, in treatment of vaginal candidosis, **124**

COHEN L, PANJA S K: Outbreak of gonorrhoea due to penicillinase-producing *N gonorrhoeae* (PPNG) in a provincial city (correspondence), **132**

COIGNAU H. *See* GORDTS B *et al*, **23**

COKER G O. *See* DAROUGAR S *et al*, **366**

COLEMAN J C. *See* VIOLA L *et al*, **59**

Conjunctiva, isolation of *H ducreyi* from, **133**

CORNELL L. *See* DAROUGAR S *et al*, **374**

CORRESPONDENCE

  BALSDON M J, LAING S R S, and TAYLOR G E: Postgonococcal cervicitis and postgonococcal urethritis, **275**

  COHEN L, PANJA S K: Outbreak of gonorrhoea due to penicillinase-producing *N gonorrhoeae* (PPNG) in a provincial city, **132**

  DE KLERK E, SHARP C A E, GEFFEN C, and ANDERSON R: False-negative reagin test results to non-agglutinating (incomplete) antibodies, **275**

  FAUR Y C, WILSON M E: Lincomycin *vs* vancomycin in New York City (NYC) medium for cultural diagnosis of gonorrhoea, **66**

  HANDSFIELD H H and FENNELL C L: Isolation of *Haemophilus ducreyi* from the conjunctiva, **133**

  JOSODIWONDO S: Penicillinase-producing *Neisseria gonorrhoeae* in Jakarta, Indonesia, **468**

  LOVEDAY C and BINGHAM J S: Changes in intravascular kininogen during the Jarisch-Herxheimer reaction in secondary syphilis, **468**

  PHILLIPS P A, HARNETT G B, and GOLLOW M M: Adenovirus type 19 and a closely related new serotype in genital infections (correspondence), **131**

  POLNIKORN N, WITOONPANICH R, VORACHIT M, VEJJAJIVA S, and VEJJAJIVA A: Penicillin concentrations in the cerebrospinal fluid after benzathine penicillin and probenecid in the treatment of syphilis, **342**

  RAMPEN F H J and VAN EVERDINGEN J J E: Inefficacy of cimetidine in condylomata acuminata, **275**

  TALBOT M D: Aetiology and management of non-specific vaginitis, **275**

  WONG G H W, STEINER B M, GRAVES S R: Lack of effect of bicarbonate on survival of *T pallidum* (Nichols) in vitro, **130**

COUFALIK E D. *See* FONTAINE E A *et al*, **321**

COUTINHO R A. *See* LUMEY L H *et al*, **402**

CRISOLOGO-VIZCONDE L B *See* CALUBIRAN O V *et al*, **231**

CSF, penicillin concentrations after benzathine penicillin and probenecid, **342**

CSONKA G. *See* DAWSON S G *et al*, **243**

DARNE J F, RIDGWAY G L, and ORIEL J D: Rosaramicin and tetracycline in the treatment of non-gonococcal urethritis: a comparison of clinical and microbiological results, **117**

DAROUGAR S, FORSEY T, OSOBA A O, DINES R J, ADELUSI B, and COKER G O: Seroepidemiological survey of chlamydial genital infection in Ibadan, Nigeria, **366**

DAROUGAR S, JONES B R, CORNELL L, TREHARNE J D, DWYER R ST C, and ARAMESH B: Chlamydial urethral infection in Teheran: a study of male patients attending an STD clinic, **374**

DAROUGAR S. *See* FORSEY T *et al*, **370**

DARWISH R. *See* AUSTIN T W *et al*, **121**

DATTANI I M, GERKEN A, and EVANS B A: Aetiology and management of non-specific vaginitis, **32**

DAUNT S O'N, KOTOWSKI K E, O'REILLY A P, and RICHARDSON A T: Ulcerative vulvitis in Reiter's syndrome: a case report, **405**

DAWSON S G, ISON C A, CSONKA G, and EASMON C S F: Male carriage of *Gardnerella vaginalis*, **243**

DAWSON S *See* ATIA W A *et al*, **52**

DAWSON S G. *See* EASMON C S F *et al*, **246**

DE HOOP D. *See* AARDOOM H A *et al*, **359**

DE KLERK E, SHARP C A E, GEFFEN C, and ANDERSON R: False-negative reagin test results to non-agglutinating (incomplete) antibodies (correspondence), **275**

DEMES P. *See* KULDA J *et al*, **394**

DEN HENGST C W. *See* VANDENBROUCKE-GRAULS C M J E *et al*, **311**

DIERICKX R *See* GORDTS B *et al*, **23**

DIN S N. *See* RATNAM A V *et al*, **355**

DINES R J. *See* DAROUGAR S *et al*, **366**

DINES R. J. *See* FORSEY T *et al*, **370**

DONALD W H: Obituary, **66**

DUARSA N W. *See* NEY R *et al*, **351**

DUNK A, JENKINS W J, and SHERLOCK S: Guillain-Barré syndrome associated with hepatitis A in a male homosexual, **269**

DURHAM J. *See* SKINNER G R B *et al*, **381**

DWYER R ST C. *See* DAROUGAR S *et al*, **374**

EASMON C S F, ISON C A, KAYE C M, TIMEWELL R M, and DAWSON S G: Pharmacokinetics of metronidazole and its principal metabolites and their activity against *Gardnerella vaginalis*, **246**

EASMON C S F. *See* DAWSON S G *et al*, **243**
EASMON C S F. *See* JACKSON D H *et al*, **334**
Editorial: statistical significances—use and misuse, **71**
ELISA for detection of antibodies to *T vaginalis* in sera and vaginal secretions, **330**
EMMERSON A M. *See* RIDGWAY G L *et al*, **236**
Ethiopia, seroepidemiological survey of chlamydial genital infection, **370**
EVANS B A. *See* DATTANI I M *et al*, **32**
EXNER A C, SHINNERS E N, PACE P J, and CATLIN B W: Auxotypes and antibacterial resistance of gonococci with differing susceptibilities to vancomycin, **166**

FAUR Y C and WILSON M E: Lincomycin versus vancomycin in New York City (NYC) medium for cultural diagnosis of gonorrhoea (correspondence), **66**
FENNELL C L. *See* HANDSFIELD H H, **133**
FENNESTAD K L, BRUUN L, and HAAHR S: Interferon in rabbit sera after inoculation with *Treponema pallidum* suspensions contaminated with PED virus, **143**
FINLEY-PRICE K G. *See* ARKO R J *et al*, **105**
FITZGERALD T J, REPESH L A, and OAKES S G: Morphological destruction of cultured cells by the attachment of *Treponema pallidum*, **1**
FITZGERALD T J. *See* OAKES S G *et al*, **220**
FITZGERALD T J. *See* REPESH L A *et al*, **211**
FIUMARA N J and CAHN T: Traumatic ulceration of the cervix: case report, **202**
Flumequine as short-term treatment for gonorrhoea, **317**
FONTAINE E A, TAYLOR-ROBINSON D, HANNA N F, and COUFALIK E D: Anaerobes in men with urethritis, **321**
FORSEY T, DAROUGAR S, DINES R J, WRIGHT D J M, and FRIEDMANN P S: Chlamydial genital infection in Addis Ababa, Ethiopia: a seroepidemiological survey, **370**
FORSEY T. *See* DAROUGAR S *et al*, **366**
FORSMANN L. *See* MILSOM I *et al*, **124**
FRIEDMANN P S. *See* FORSEY T *et al*, **370**
FROM E: *See* JENSEN J R, **18**
FULLER A. *See* SKINNER G R B *et al*, **381**

Gardnerella vaginalis, activity of metronidazole against, **246**
G vaginalis infection associated with balanoposthitis, **127**
G vaginalis, male carriage of, **243**
G vaginalis vaginitis, aetiology and management, **32**, **275**
GARNER M F. *See* NEY R *et al*, **351**
GEARY I. *See* KINGHORN G R *et al*, **127**
GEFFEN C. *See* DE KLERK E *et al*, **275**
GERKEN A. *See* DATTANI I M *et al*, **32**
GODDARD A D. *See* MALLINSON H *et al*, **36**
GOH A J. *See* SNG E H *et al*, **239**
GOH B T, MORGAN-CAPNER P, and LIM K S: Chlamydial screening of pregnant women in a sexually transmitted diseases clinic, **327**
GOH C L. *See* RAJAN V S *et al*, **314**
GOH C L. *See* THIRUMOORTHY T *et al*, **308**
GOLDMEIER D and JOHNSON A: Does psychiatric illness affect the recurrence rate of genital herpes?, **40**
GOLLOCK J M, SLATFORD K, and HUNTER J M: Scissor excision of anogenital warts, **400**
GOLLOW M M. *See* PHILLIPS P A *et al*, **131**
Gonococci, induction of phenotypic resistance to killing by human serum by human genital secretions, **363**
Gonococci, penicillinase-producing, in Zambia, **29**
Gonococci, rapidity and reliability of identification by coagglutination, **109**
Gonococci, susceptibility to vancomycin, auxotypes and antibacterial resistance, **166**
Gonorrhoea, epidemiological treatment of female sexual partners, **113**
Gonorrhoea in women, comparison of rosoxacin and spectinomycin treatments, **231**
Gonorrhoea, pharyngeal, β-lactamase-producing, treatment of, **101**
Gonorrhoea, treatment with flumequine, **317**
Gonorrhoea, uncomplicated in women, single 1·5 g oral talampicillin for, **180**
Gonorrhoea, vancomycin versus lincomycin in New York City medium for, **66**
GORDTS B, VANHOOF R, HUBRECHTS J M, DIERICKX R, COIGNAU H, and BUTZLER J P: In-vitro activity of 21 antimicrobial agents against *Neisseria gonorrhoeae* in Brussels, **23**
Granuloma inguinale, vaginal bleeding in, **200**
GRAVES S R. *See* WONG G H W *et al*, **130**, **139**
GREENFIELD C. *See* ATIA W A *et al*, **52**
GROVE D I: Strongyloidiasis: is it transmitted from husband to wife? **271**
GRUNINGER R P. *See* KHAN M Y *et al*, **228**
Guillain-Barrè syndrome associated with hepatitis A in a male homosexual, **269**

HAAHR S. *See* FENNESTAD K L *et al*, **143**
Haemophilus ducreyi, characteristics of, **239**
H ducreyi, isolation from conjunctiva, **133**
HAFIZ S. *See* KINGHORN G R *et al*, **377**
HANDSFIELD H H and FENNELL C L: Isolation of *Haemophilus ducreyi* from the conjuctiva (correspondence), **133**
HANNA N F. *See* FONTAINE E A *et al*, **321**
HANNA N F. *See* STREET D A *et al*, **330**
HARNETT G B. *See* PHILLIPS P A *et al*, **131**
Harrison Lecture 1981: The international venereological scene as viewed by Harrison and St Mary's, **72**
HARTLEY C E. *See* SKINNER G R B *et al*, **381**
Hepatitis A associated with Guillain-Barrè syndrome in a male homosexual, **269**
Hepatitis HBeAg-positive homosexuals with chronic liver disease, cell-mediated immunity in, **59**
Herpes, genital, incidence of HSV-1 and HSV-2 in Sheffield, **44**
Herpes, genital, preparation and immunogenicity of AcNFU$_1$ (S$^-$) MRC vaccine, **381**
Herpes, genital, recurrence rate and psychiatric illness, **40**
Herpes simplex proctitis and aseptic meningitis, **52**
Herpes simplex virus type 2, confirmation by simple complement-fixation test, **48**
HINDER S M. *See* JACKSON D H *et al*, **334**
HIRA S K. *See* RATNAM A V *et al*, **29**, **355**
Homosexually acquired VD, social factors compared in Sweden and Australia, **263**
Homosexual, Guillain-Barrè syndrome associated with hepatitis A in, **269**
Homosexuals, HBeAg-positive, with chronic liver disease, cell-mediated immunity in, **59**

Homosexuals, screening for syphilis in Amsterdam bars and saunas, **402**
HORNER T. *See* LAVERY H A *et al*, **196**
HUBRECHTS J M. *See* GORDTS B *et al*, **23**
HUNTER J M. *See* GOLLOCK J M *et al*, **400**

ILIAS M I. *See* SOENDJOJO A *et al*, **149**
India, STD in, **338**
Indonesia, penicillinase-producing *N gonorrhoeae* in, **351**
Indonesia, treponemal serology on Bali Island, **351**
Interferon in rabbit sera after inoculation with PED virus-contaminated *T pallidum*, **143**
ISERIEF C O A. *See* AARDOOM H A *et al*, **359**
ISON C A. *See* DAWSON S G *et al*, **243**
ISON C A. *See* EASMON C S F *et al*, **246**
Italy, role played by, in history of syphilis, **92**
IUVDT—South-east Asian and Western Pacific regional conference, **273**

JACKSON D H, HINDER S M, STRINGER J, and EASMON C S F: Carriage and transmission of group B streptococci among STD clinic patients, **334**
Japan, antibiotic sensitivity patterns of *N gonorrhoeae*, **305**
Jarisch-Herxheimer reaction, changes in intravascular kininogen in, in secondary syphilis, **468**
JENKINS W J. *See* DUNK A *et al*, **269**
JENSEN J R and FROM E: Alterations in T lymphocytes and T-lymphocyte subpopulations in patients with syphilis, **18**
JENSEN J R, JORGENSEN A S, and THESTRUP-PEDERSEN K: Depression of natural killer cell activity by syphilitic serum and immune complexes, **298**
JERVE F. *See* QVIGSTAD E *et al*, **182**
JOHNSON A. *See* GOLDMEIER D, **40**
JONES B M. *See* KINGHORN G R *et al*, **127**
JONES B R. *See* DAROUGAR S *et al*, **374**
JONES L W. *See* MALISON M D *et al*, **62**
JORGENSEN A S. *See* JENSEN J R *et al*, **298**
JOSODIWONDO S: Penicillinase-producing *Neisseria gonorrhoeae* in Jakarta, Indonesia (correspondence), **408**
JUVAKOSKI T. *See* KANERVA L *et al*, **188**

KANERVA, KOUSA M, NIEMI K-M, LASSUS A, JUVAKOSKI T, and LAUHARANTA J: Ultrahistopathology of balanitis circinata, **188**
KAYE C M. *See* EASMON C S F *et al*, **246**
KHAN M Y, SIDDIQUI Y, and GRUNINGER R P: Comparative in-vitro activity of selected new *β*-lactam antimicrobials against *Neisseria gonorrhoeae*, **228**
KINGHORN G R, HAFIZ S, and McENTEGART M C: Pathogenic microbial flora of genital ulcers in Sheffield, **377**
KINGHORN G R, JONES B M, CHOWDHURY F H, and GEARY I: Balanoposthitis associated with *Gardnerella vaginalis* infection in men, **127**
KINGHORN G R. *See* BARTON I G *et al*, **44**
KOK J. *See* LUMEY L H *et al*, **402**
KOTOWSKI K E. *See* DAUNT S O'N *et al*, **405**
KOUSA M. *See* KANERVA L *et al*, **188**
KULDA J, VOJTECHOVSKA M, TACHEZY J, DEMES P, and KUNZOVA E: Metronidazole resistance of *Trichomonas vaginalis* as a cause of treatment failure in a woman with trichomoniasis, **394**
KUNZOVA E. *See* KULDA J *et al*, **394**

LAING S R S. *See* BALSDON M J, **275**
LASSUS A. *See* KANERVA L *et al*, **188**
LATIF A S: Thiamphenicol in the treatment of chancroid in men, **54**
LAUHARANTA J. *See* KANERVA L *et al*, **188**
LAVERY H A, HORNER T, and ROBERTS S D: Rheumatoid arthritis, ankylosing spondylitis, and Reiter's syndrome occurring simultaneously: case report, **196**
LE FROCK J L. *See* CHAN J K *et al*, **292**
LIM A L. *See* SNG E H *et al*, **239**
LIM K S. *See* GOH B T *et al*, **327**
LIMSON B M. *See* CALUBIRAN O V *et al*, **231**
Lincomycin versus vancomycin in New York City (NYC) medium for gonorrhoea, **66**
LINDBERG M, RINGERTZ O, SANDSTRÖM E: Treatment of pharyngeal gonorrhoea due to *β*-lactamase-producing gonococci, **101**
LINDENSCHMIDT E-G: *See* MÜLLER F, **12**
Liver disease, chronic, cell-mediated immunity in HBeAg-positive homosexuals, **59**
LOVEDAY C and BINGHAM J S: Changes in intravascular kininogen during the Jarisch-Herxheimer reaction in secondary syphilis (correspondence), **468**
LUMEY L H, KOK J, and COUTINHO R A: Screening for syphilis among homosexual men in two bars and saunas in Amsterdam: an effective method of case finding, **402**

McENTEGART M C. *See* KINGHORN G R *et al*, **377**
McMILLAN A. *See* STREET D A *et al*, **330**
McMILLAN A. *See* YOUNG H, **109**
MAELAND J A. *See* SVINDLAND H B *et al*, **317**
MALISON M D, MORRIS R, and JONES L W: Autogenous vaccine therapy for condyloma acuminatum: a double-blind controlled study, **62**
MALLINSON H, ARYA O P, and GODDARD A D: Quantitative study of *Chlamydia trachomatis* in genital infection, **36**
MARTIN P M V, PATEL P V, PARSONS N J, and SMITH H: Induction in gonococci of phenotypic resistance to killing by human serum by human genital secretions, **363**
MARTIN P M V, PATEL P V, PARSONS N J, and SMITH H: Induction of phenotypically determined resistance of *Neisseria gonorrhoeae* to human serum by sera from patients with gonorrhoea, **302**
Martin-Lewis medium, inhibition of *N gonorrhoeae* isolates, **96**
MAY J T. *See* ARSENAKIS M, **48**
MELLING J. *See* SKINNER G R B *et al*, **381**
Meningitis, aseptic, and herpes simplex proctitis, **52**
Metronidazole, failure of 1-g single dose for trichomoniasis, **121**
Metronidazole, pharmacokinetics and principal metabolites of, activity against *G vaginalis*, **246**
Metronidazole, rectal suppositories in treatment of *T vaginalis*, **257**
Metronidazole resistance of *T vaginalis* giving treatment failure, **394**
MICHEL M F. *See* AARDOOM H A *et al*, **359**
MILLER M A, ANDERSON P, PARKER J W, ROHRER H H: Inhibition of *N gonorrhoeae* isolates by Martin-Lewis medium epidemiology, susceptibility profile, and plasmid analysis, **96**

MILSOM I and FORSSMAN L: Treatment of vaginal candidosis with a single 500 mg clotrimazole pessary, **124**

MIZUGUCHI Y. *See* YOSHIDA S-I *et al*, **305**

MOHANTY K C and ROY R B: Treatment of uncomplicated gonorrhoea in women with talampicillin in a single oral 1·5 g dose, **180**

MORGAN-CAPNER P. *See* GOH B T *et al*, **327**

MORRIS R. *See* MALISON M D *et al*, **62**

MOSS V. *See* RIDGWAY G L *et al*, **236**

MOSTRATOS A. *See* AL-QUDAH A A, **281**

MULENGA R C. *See* RATNAM A V *et al*, **29**, **355**

MÜLLER F and LINDENSCHMIDT E-G: Demonstration of specific 19S(IgM) antibodies in untreated and treated syphilis: comparative studies of the 19S(IgM) FTA test, the 19S(IgM) TPHA test, and the solid-phase haemadsorption assay, **12**

MUMTAZ G. *See* RIDGWAY G L *et al*, **236**

MURRAY-LYON I M. *See* VIOLA L *et al*, **59**

MURUGAN S, VENKATRAM K, and RENGANATHAN P S: Vaginal bleeding in granuloma inguinale: case reports, **200**

NAJEM S. *See* BARTON I G *et al*, **44**

Neisseria gonorrhoeae, activity of new β-lactam antimicrobials against, **228**

N gonorrhoeae, antibiotic sensitivity patterns in Japan, **305**

N gonorrhoeae antigen, detection with a solid-phase enzyme immunoassay, **359**

N gonorrhoeae, ceftriaxone for treatment of, **314**

N gonorrhoeae, effects of tampon components on growth and dissemination of, **105**

N gonorrhoeae, induction of resistance to human serum by serum from patients with gonorrhoea, **302**

N gonorrhoeae, in-vitro activity of 21 antibiotics in Brussels, **23**

N gonorrhoeae isolated from infections in pre-penicillin era: auxotypes and antibacterial drug resistances, **158**

N gonorrhoeae isolates, inhibition by Martin-Lewis medium, **96**

N gonorrhoeae, penicillinase-producing, in a provincial city, **132**

N gonorrhoeae, penicillinase-producing, in Jakarta, Indonesia, **468**

N gonorrhoeae, penicillinase-producing ophthalmia neonatorum in Singapore, **308**

N gonorrhoeae, penicillinase-producing, prostatitis due to, **311**

N gonorrhoeae, penicillinase-producing, single oral dose of cefaclor for, **176**

N gonorrhoeae, penicillinase-producing, treatment of pharyngeal infection, **101**

New York City medium, rapidity and reliability of gonococcal identification by coagglutination after culture on, **109**

NEY R, GARNER M F, BACKHOUSE J L, DUARSA N W, BREGUET D, and BREGUET G: Treponemal serology on Bali Island, Indonesia, **351**

NIEMI K-M. *See* KANERVA L *et al*, **188**

Nigeria, seroepidemiological survey of chlamydial genital infection in, **366**

Non-gonococcal urethritis, comparison of rosaramicin and tetracycline treatments, **117**

Non-specific genital infection, epidemiological treatment of female sexual partners, **113**

OAKES S G, REPESH L A, POZOS R S, and FITZGERALD T J: Electrophysiological dysfunction and cellular disruption of sensory neurones during incubation with *Treponema pallidum*, **220**

OAKES S G. *See* FITZGERALD T J *et al*, **1**

OAKES S G. *See* REPESH L A *et al*, **211**

OBITUARY
  W H DONALD, **66**

O'CONNOR B H. *See* ADLER M W *et al*, **151**

Ophthalmia neonatorum, penicillinase-producing gonococcal, **308**

ORIEL J D. *See* DARNE J F *et al*, **117**

ORIEL J D. *See* RIDGWAY G L *et al*, **236**

O'REILLY A P. *See* DAUNT S O'N *et al*, **405**

OSOBA A O. *See* DAROUGAR S *et al*, **366**

PACE P J. *See* EXNER A C *et al*, **166**

PANJA S K. *See* COHEN L, **132**

PANJA S K: Treatment of trichomoniasis with metronidazole rectal suppositories, **257**

PARADINAS F. *See* VIOLA L *et al*, **59**

PARKER J W. *See* MILLER M A *et al*, **96**

PARSONS N J. *See* MARTIN P M W *et al*, **302**, **363**

PATEL M I. *See* RATNAM A V *et al*, **29**

PATEL P V. *See* MARTIN P M W *et al*, **302**, **363**

PATTISON F L M. *See* AUSTIN T W *et al*, **121**

Pelvic inflammatory disease, morbidity associated with, **151**

Penicillin concentrations in CSF after benzathine penicillin and probenecid, **342**

PENN C W and CLAY J C: Comparison of the morphology, antigenicity, and pathology of Nichols and Birmingham isolates of *Treponema pallidum*, **286**

PHILLIPS P A, HARNETT G B, and GOLLOW M M: Adenovirus type 19 and a closely related new serotype in genital infections (correspondence), **131**

Pleural effusion disease virus contaminating *T pallidum*, interferon in rabbit sera after inoculation with, **143**

Poland, symposium on trichomoniasis, and medicine in, **56**

POLNIKORN N, WITOONPANICH R, VORACHIT M, VEJJAJIVA S, and VEJJAJIVA A: Penicillin concentrations in the cerebrospinal fluid after benzathine penicillin and probenecid in the treatment of syphilis (correspondence), **342**

POTTER C W. *See* BARTON I G *et al*, **44**

POZOS R S. *See* OAKES S G *et al*, **220**

POZOS R S. *See* REPESH L A *et al*, **211**

Prostatitis due to penicillinase-producing *N gonorrhoeae*, **311**

QVIGSTAD E, SKAUG K, JERVE F, VIK I S S, ULSTRUP J C: Therapeutic abortion and *Chlamydia trachomatis* infection, **182**

RAHARDJO D. *See* SOENDJOJO A *et al*, **149**

RAJAN V S, SNG E H, THIRUMOORTHY T, and GOH C L: Ceftriaxone in the treatment of ordinary and penicillinase-producing strains of *Neisseria gonorrhoeae*, **314**

RAJAN V S. *See* SNG E H *et al*, **239**

RAJAN V S. *See* THIRUMOORTHY T *et al*, **308**

RALPH E D. *See* AUSTIN T W *et al*, **121**

RAMPEN F H J and VAN EVERDINGEN J J E: Inefficacy of cimetidine in condylomata acuminata (correspondence), **275**

RASHEED J K. *See* ARKO R J *et al*, **105**

RATNAM A V, DIN S N, HIRA S K, BHAT G J, WACHA D S O, RUKMINI A, and MULENGA R C: Syphilis in pregnant women in Zambia, **355**

RATNAM A V, PATEL M I, MULENGA R C, HIRA S K: Penicillinase-producing gonococcal strains in Zambia: observations on treatment failures, **29**

RATNATUNGA C S. *See* ATIA W A *et al*, **52**

Reiter haemagglutination test, **281**

Reiter's syndrome occurring simultaneously with rheumatoid arthritis, and ankylosing spondylitis, **196**

Reiter's syndrome, ulcerative vulvitis in, **405**

RENGANATHAN P S. *See* MURUGAN S *et al*, **200**

REPESH L A, FITZGERALD T H, OAKES S G, and POZOS R S: Scanning electron microscopy of the attachment of *Treponema pallidum* to nerve cells in vitro, **211**

REPESH L A. *See* FITZGERALD T J *et al*, **1**

REPESH L A. *See* OAKES S G *et al*, **220**

REYN A. *See* CATLIN B W, **158**

RICHARDSON A T. *See* DAUNT S O'N *et al*, **405**

RIDGWAY G L, MOSS V, MUMTAZ G, ATIA W, EMMERSON A M, and ORIEL J D: Provision of a chlamydial culture service to a sexually transmitted diseases clinic, **236**

RIDGWAY G L. *See* DARNE J F *et al*, **117**

RINGERTZ O. *See* LINDBERG M *et al*, **101**

Rheumatoid arthritis occurring simultaneously with ankylosing spondylitis and Reiter's syndrome, **196**

ROBERTS S D. *See* LAVERY H A *et al*, **196**

ROHRER H H. *See* MILLER M A *et al*, **96**

Rosaramicin and tetracycline treatments compared in non-gonococcal urethritis, **117**

Rosoxacin and spectinomycin treatments compared in gonorrhoea in women, **231**

ROSS M W: Social factors in homosexually acquired venereal disease: comparison between Sweden and Australia, **263**

ROY R B. *See* MOHANTY K C, **180**

ROZENBERG-ARSKA M. *See* VANDENBROUCKE-GRAULS C M J E *et al*, **311**

RUKMINI A. *See* RATNAM A V *et al*, **355**

SANDSTRÖM E. *See* LINDBERG M *et al*, **101**

SCHELL R F. *See* CHAN J K *et al*, **292**

SCHOFIELD C B S: Higher medical training in venereology, **86**

SCHOFIELD C B S: Some factors affecting the incubation period and duration of symptoms of urethritis in men, **184**

SELKON J B. *See* SPROTT M S *et al*, **147**

SHARP C A E. *See* DE KLERK E *et al*, **275**

Sheffield, incidence of HSV-1 and HSV-2 in, **44**

Sheffield, pathogenic microbial flora of genital ulcers in, **377**

SHERLOCK S. *See* DUNK A *et al*, **269**

SHINNERS E N. *See* EXNER A C *et al*, **166**

SIDDIQUI Y. *See* KHAN M Y *et al*, **228**

Singapore, penicillinase-producing gonococcal ophthalmia neonatorum, **308**

SKAUG K. *See* QVIGSTAD E *et al*, **182**

SKINNER G R B, WOODMAN C B J, HARTLEY C E, BUCHAN A, FULLER A, DURHAM J, SYNNOTT M, CLAY J C, MELLING J, WIBLIN C, and WILKINS J: Preparation and immunogenicity of vaccine Ac NFU$_1$ (S$^-$) MRC towards the prevention of herpes genitalis in man, **381**

SLATFORD K. *See* GOLLOCK J M *et al*, **400**

SMITH E A. *See* AUSTIN T W *et al*, **121**

SMITH H. *See* MARTIN P M W *et al*, **302, 363**

SNG E H, LIM A M, RAJAN V S, and GOH A J: Characteristics of *Haemophilus ducreyi*: a study, **239**

SNG E H. *See* RAJAN V S *et al*, **314**

SOENDJOJO A, BOEDISANTOSO M, ILIAS M I, and RAHARDJO D: Syphilis d'emblée due to blood transfusion: case report, **149**

SOLIMAN M A, ACKERS J P, and CATTERALL R D: Isoenzyme characterisation of *Trichomonas vaginalis*, **250**

Spectinomycin and rosoxacin treatments compared in gonorrhoea in women, **231**

SPROTT M S, SELKON J B, TURNER R H: Evaluation of the role of the *Treponema pallidum* immobilisation test in Britain, **147**

Sri Lanka, STD in, **338**

STD in India and Sri Lanka, **338**

STD—Sixth National Conference of the Indian Association, **340**

STD, television programme on, consulting patterns after, **259**

STD, world and Latin American congress on, **204**

STEINER B M. *See* WONG G H W *et al*, **130, 139**

STOLZ E. *See* AARDOOM H A *et al*, **359**

STREET D A, TAYLOR-ROBINSON D, ACKERS J P, HANNA N F, and McMILLAN A: Evaluation of an enzyme-linked immunosorbent assay for the detection of antibody to *Trichomonas vaginalis* in sera and vaginal secretions, **330**

Streptococci, group B, carriage and transmission among STD clinic patients, **334**

STRINGER J. *See* JACKSON D H *et al*, **334**

Strongyloidiasis, husband-to-wife transmission, **271**

SVARVA P L. *See* SVINDLAND H B *et al*, **317**

SVINDLAND H B, SVARVA P L, and MAELAND J A: Quinolone derivative, flumequine, as short-term treatment for gonorrhoea, **317**

Sweden and Australia, comparison of social factors in homosexually acquired VD, **263**

SYNNOTT M. *See* SKINNER G R B *et al*, **381**

Syphilis, alterations in T lymphocytes in patients with, **18**

Syphilis d'emblée due to blood transfusion, **149**

Syphilis in pregnant women in Zambia, **355**

Syphilis, penicillin concentration in CSF after benzathine penicillin and probenecid, **342**

Syphilis, role played by Italy in history of, **92**

Syphilis screening among homosexuals in Amsterdam bars and saunas, **402**

Syphilis, secondary, changes in intravascular kininogen during Jarisch-Herxheimer reaction, **468**

Syphilis serology, comparison of FTA(IgM), TPHA(IgM), and solid-phase haemadsorption tests, **12**

Syphilis serology: false-negative reagin test results to non-agglutinating (incomplete) antibodies, **275**

Syphilis serology on Bali Island, Indonesia, **351**

Syphilis serology: Reiter haemagglutination test, **281**

Syphilis serology, TPI test in Britain, **147**

Syphilis serum, depression of natural killer cell activity by, **298**

TACHEZY J. *See* KULDA J *et al,* **394**
Talampicillin, single 1·5 g oral dose for uncomplicated gonorrhoea in women, **180**
TALBOT M D: Aetiology and management of non-specific vaginitis (correspondence), **275**
Tampon components, effects on growth and dissemination of *N gonorrhoeae,* **105**
TAYLOR G E. *See* BALSDON M J, **275**
TAYLOR-ROBINSON D. *See* FONTAINE E A *et al,* **321**
TAYLOR-ROBINSON D. *See* STREET D A *et al,* **330**
Teheran, chlamydial urethral infection in male STD clinic patients, **374**
Tetracycline and rosaramicin treatments compared in non-gonococcal urethritis, **117**
THESTRUP-PEDERSEN K. *See* JENSEN J R *et al,* **298**
Thiamphenicol in the treatment of chancroid, **54**
THIRUMOORTHY T, RAJAN V S, and GOH C L: Pencillinase-producing *Neisseria gonorrhoeae* ophthalmia neonatorum in Singapore, **308**
THIRUMOORTHY T. *See* RAJAN V S *et al,* **314**
TIMEWELL R M. *See* EASMON C S F *et al,* **246**
TORRES C A. *See* CALUBIRAN O V *et al,* **231**
TORRES C A. *See* TUPASI T E *et al,* **176**
TREHARNE J D. *See* DAROUGAR S *et al,* **374**
Treponema pallidum, attachment to nerve cells, **211**
T pallidum, comparison of Nichols and Birmingham isolates, **286**
T pallidum contaminated with PED virus, interferon in rabbit sera after inoculation with, **143**
T pallidum, destruction of cultured cells by attachment of, **1**
T pallidum, dysfunction and cellular disruption of sensory neurones during incubation with, **220**
T pallidum, effect of anaerobic and microaerophilic conditions of extraction and incubation on survival of, **139**
T pallidum immobilisation test in Britain, **147**
T pallidum, lack of effect of bicarbonate on in-vitro survival, **130**
T pertenue, lack of correlation of passive transfer of resistance and mitogenic responses to, **292**
Trichomonas vaginalis, evaluation of ELISA for detection of antibody in sera and vaginal secretions, **330**
T vaginalis, failure of 1-g single dose treatment, **121**
T vaginalis, isoenzyme characterisation of, **250**
T vaginalis, treatment failure due to metronidazole resistance, **394**
T vaginalis, treatment with metronidazole rectal suppositories, **257**
Trichomoniasis—international symposium in Poland, **56**
TUPASI T E, CALUBIRAN O V, and TORRES C A: Single oral dose of cefaclor for the treatment of infections with penicillinase-producing strains of *Neisseria gonorrhoeae,* **176**
TUPASI T E. *See* CALUBIRAN O V *et al,* **231**
TURNER R H. *See* SPROTT M S *et al,* **147**

Ulcers, genital, pathogenic microbial flora of, in Sheffield, **377**
ULSTRUP J C. *See* QVIGSTAD E *et al,* **182**
URABE S. *See* YOSHIDA S-I *et al,* **305**
Urethritis, factors affecting incubation period and duration of symptoms, **184**
Urethritis, postgonococcal, and postgonococcal cervicitis, **275**
Urethritis, male, anaerobes in, **321**

Vaginitis, non-specific, aetiology and management of, **32, 275**
Vaginitis, non-specific: clinic diagnosis: a practical guide, **387**
Vancomycin, gonococcal susceptibility, auxotypes and antibacterial resistance, **166**
Vancomycin versus lincomycin in New York City (NYC) medium for gonorrhoea, **66**
VANDENBROUCKE-GRAULS C M J E, ROZENBERG-ARSKA M, DEN HENGST C W, and VERHOEF J: Prostatitis due to penicillinase-producing *Neisseria gonorrhoeae:* case reports, **311**
VAN EVERDINGEN J J E. *See* RAMPEN F H J, **275**
VANHOOF R. *See* GORDTS B *et al,* **23**
Venereology, higher medical training in, **86**
VEJJAJIVA A. *See* POLNIKORN N *et al,* **342**
VEJJAJIVA S. *See* POLNIKORN N *et al,* **342**
VENKATRAM K. *See* MURUGAN S *et al,* **200**
VERHOEF J. *See* VANDENBROUCKE-GRAULS C M J E *et al,* **311**
VIK I S S. *See* QVIGSTAD E *et al,* **182**
VIOLA L, BARRISON I G, PARADINAS F, COLEMAN J S, and MURRAY-LYON I M: Cell-mediated immunity in HBeAg-positive homosexuals in chronic liver disease, **59**
VOJTECHOVASKA M. *See* KULDA J *et al,* **394**
VORACHIT M. *See* POLNIKORN N *et al,* **342**
Vulvitis, ulcerative, in Reiter's syndrome, **405**

WACHA D S O. *See* RATNAM A V *et al,* **355**
Warts, anogenital, scissor excision, **400**
Warts, genital autogenous vaccine therapy for, **62**
Warts, genital, inefficacy of cimetidine in, **275**
WAUGH M A: Role played by Italy in the history of syphilis, **92**
WIBLIN C. *See* SKINNER G R B *et al,* **381**
WILKINS J. *See* SKINNER G R B *et al,* **381**
WILLCOX R R: IV Harrison Lecture 1981: the international venereological scene as viewed by Harrison and St Mary's, **72**
WILSON M E. *See* FAUR Y C, **66**
WITOONPANICH R. *See* POLNIKORN N *et al,* **342**
WONG G H W, STEINER B M, GRAVES S R: Effects of anaerobic and microaerophilic conditions of extraction and incubation on the survival of *Treponema pallidum* in vitro, **139**
WONG G H W, STEINER B M, and GRAVES S R: Lack of effect of bicarbonate on survival of *T pallidum* (Nichols) in vitro (correspondence), **130**
WONG K H. *See* ARKO R J *et al,* **105**
WOODMAN C B J. *See* SKINNER G R B *et al,* **381**
WRIGHT D G M. *See* FORSEY T *et al,* **370**

YOSHIDA S-I, URABE S, and MIZUGUCHI Y: Antibiotic sensitivity patterns of penicillinase-positive and penicillinase-negative strains of *Neisseria gonorrhoeae* in Fukuoka, Japan, **305**
YOUNG H and McMILLAN A: Rapidity and reliability of gonococcal identification by coagglutination after culture on modified New York City Medium, **109**

Zambia, penicillinase-producing gonococci in, **29**
Zambia, syphilis in pregnant women in, **355**

Br J Vener Dis 1982;58:418-23

Index to subjects of articles abstracted, 1982

**Indicates that only the title is given.*

Acridine orange stain, evaluation in detection of *T vaginalis*, 279*
Acycloguanosine inhibition of herpes simplex virus transformed and non-transformed cells, 136*
Alopecia, syphilitic, 345*
Amphotericin B, *Candida* spp sensitivity to, 136*
Ampicillin treatment of *N gonorrhoeae*, study in rabbits, 135*
Amyl nitrite alters T lymphocytes in homosexuals?, 349
Arsenotherapy of early syphilis, 345*

Behçet's disease, association with HLA-B5 in Mexican Mestizo population, 70*
Behçet's disease, sacroiliitis in, 280*
Boric acid powder in treatment of vulvovaginal candidiasis, 209*
Borrelia turicatae, helical conformation of, 67

Campylobacter foetus spp *jejuni* associated with Reiter's syndrome, 136*
Canada, penicillinase-producing *N gonorrhoeae*, 210*
Canada, penicillinase-producing *N gonorrhoeae*, molecular and phenotypic characterisation of, 67*
Candida albicans, adherence to human buccal epithelial cells after growth on different carbon sources, 280*
C albicans antigens, responses of human peripheral lymphocytes, 136*
C albicans, blastospores and hyphae, tissue response in mice, 136*
C albicans, characterisation of major cytoplasmic antigen, 280*
C albicans, corticosteroid binding protein and endogenous ligand in, 209*
C albicans infections, ultrastructure of, 280*
C albicans, relations with other *Candida* species, immunology of, 280*
Candida spp, sensitivity to 5,fluorocytosine, amphotericin B and imidazoles, 136*
Candidiasis, mucosal, in previously healthy homosexuals, 348
Candidiasis, precipitation tests in, 209*
Candidiasis, vulvovaginal, treatment with boric acid powder, 209
Carbamazepine *vs* high dosage intravenous penicillin therapy for tabetic lightning pains, 206*
Carbon dioxide laser treatment for genital warts, 280, 280*
Cefamandole, lack of activity against *C trachomatis*, 348*
Cefemoxime, activity against *N gonorrhoeae*, 279*
Cefoperazone, activity against *N gonorrhoeae*, 279*
Cefotaxime treatment for gonorrhoea, 208*
Cefoxitin, activity against *N gonorrhoeae*, 279*
Cefoxitin, lack of activity against *C trachomatis*, 348*
Ceftriaxone for uncomplicated gonorrhoea in men, 279*
Cefuroxime, activity against *N gonorrhoeae*, 279*
Cefuroxime for gonorrhoea and *C trachomatis* infections in children, 208
Cefuroxime, lack of activity against *C trachomatis*, 348*
Cephalosporins, comparative activity against *N gonorrhoeae* and *N meningitidis* strains, 135*
Cervical atypia and STD, 349*
Cervical atypia, *C trachomatis* and herpes simplex virus IgA in cervical secretions in patients with, 279*
Chancroid, an epidemic of 587 cases, 349*
Chancroid, treatment of, 210
Chancroid, treatment with erythromycin, 210*
Chlamydia associated with infant pneumonitis, 210*
Chlamydia, growth in emetine-treated McCoy cells, 348*
Chlamydiae, role in genitourinary disease, 279*
Chlamydia trachomatis, activity of clindamycin against, 208*
C trachomatis and gonorrhoea in children, treatment with cefuroxime, 208
C trachomatis and herpes simplex virus IgA in cervical secretions of patients with cervical atypia, 279*
C trachomatis and *N gonorrhoeae* prevalence in two different female populations, 68*
C trachomatis, cultural method for large-scale screening, 136
C trachomatis, effect of analgesics and lubricants on isolation of, 347*
C trachomatis, effect of cycloheximide on, in McCoy cells, 69*
C trachomatis, efficacy of treatments in uncomplicated infections in women, 348*
C trachomatis in lower genital tract infection, 69
C trachomatis in mothers and infants, 68*
C trachomatis infection, mouse model: progesterone for, 279*
C trachomatis infections, cellular immune response in, 208*
C trachomatis isolated from fallopian tubes in acute salpingitis, 208
C trachomatis, isolation from urethra and prostatic fluid in acute urethritis, 208*
C trachomatis, isolation from women with urethral syndrome, 208*
C trachomatis, lack of activity of cefoxitin, cefamandole, cefuroxime, and piperacillin against, 348*
C trachomatis, maternal infections, prematurity and perinatal mortality in, 348
C trachomatis, microtest for, 68*
C trachomatis, monoclonal antibody specificities and antigen characterisation, 348*
C trachomatis, *M hominis*, and *N gonorrhoeae* in patients with acute pelvic inflammatory disease, 208*
C trachomatis, pelvic infection after elective abortion, 348*
C trachomatis, polypeptide composition of, 68
C trachomatis, recovery from vaginal tampons, 348*

Chlamydial aetiology of sclerosing lymphangitis of penis? **208***
Chlamydial genital infections, manifestations and management, **136***
Chlamydial infection of female genital tract; review of Scandinavian studies, **135***
Chlamydial infections, epidemiology and implications for trachoma therapy, **135***
Chlamydial infections in Italy, **348***
Chlamydial genital infections in male guinea pigs, association with cystitis, **208***
Chlamydial infections, sexually transmitted, public health implications, **137***
Chlamydial urethritis, one-week treatment with doxycycline and tetracycline, **69***
Clindamycin activity against *C trachomatis*, **208***
Co-trimoxazole, treatment of uncomplicated gonorrhoea, **347***
CSF penicillin levels during treatment of latent syphilis, **277**
CSF, treponemacidal level of penicillin in, **134***
CSF Wassermann reaction in Denmark 1971-79, **277**
Cycloheximide, effect of, on *C trachomatis* in McCoy cells, **69***
Cystitis associated with chlamydial genital tract infections in male guinea pigs, **208***
Cytomegalovirus associated with infant pneumonitis, **210***
Cytomegalovirus, recovery from vaginal tampons, **348***

Denmark, profile of neurosyphilis, **277**
Dermatitis, contact, from gonococcal discharge, **347***
Doxycycline, one-week treatment in chlamydial urethritis, **69***
Doxycycline treatment for non-gonococcal urethritis, **135***
Doxycycline three-day treatment for gonorrhoea, **135***

ELISA to detect antibodies to gonorrhoea using whole cells, **207***
ELISA with polyvalent gonococcal antigen, **347***
Entamoeba histolytica and *T vaginalis*, metronidazole metabolism in cultures of, **209**
Enteric protozoa and helminths, sexual transmission in a VD clinic population, **137**
Erythromycin for treatment of chancroid, **210***

5,fluorocytosine, *Candida* spp sensitivity to, **136***
FTA-ABS double-staining procedure, **134***

Gardnerella vaginalis, rapid identification in non-purulent vaginitis, **138**
G vaginalis vaginitis, treatment of, **70***
General paralysis of the insane, **277***
Gentamicin and spectinomycin compared in penicillin-resistant gonococci, **135***
Gonococcal and non-gonococcal urethritis, application of limulus test device in rapid evaluation of, **207**
Gonococcal antigen, polyvalent, ELISA with, **347***
Gonococcal arthritis, patterns of, **279***
Gonococcal capsule electron microscopy, **135***
Gonococcal ophthalmia with little or no inflammatory response, **67***
Gonococcal outer membrane proteins, degradation by human neutrophil lysosomal proteases, **207***
Gonococcal pilus vaccine, antigenicity and inhibition of attachment, **207***
Gonococcal urethritis, office management of, **135***
Gonococcal urethritis, rapid presumptive diagnosis of, with limulus lysate test kit, **347***
Gonococcal urethritis with bilateral tysonitis and periurethral abscess, **134***
Gonococci, penicillin-resistant, comparison between gentamicin and spectinomycin, **135***
Gonorrhoea and *C trachomatis* in children, treatment with cefuroxime, **208**
Gonorrhoea and syphilis, epidemiology update, **137***
Gonorrhoea, contact dermatitis from discharge, **347***
Gonorrhoea, diagnosis of, **67***
Gonorrhoea, ELISA to detect antibodies, using whole cells, **207***
Gonorrhoea, fetal, deep tissue infection occurring in utero, **346**
Gonorrhoea in men, ceftriaxone in, **279***
Gonorrhoea in men, piperacillin *vs* penicillin G treatments, **279***
Gonorrhoea, pathogenesis of, **279***
Gonorrhoea, pharyngeal, **279***
Gonorrhoea, male, evaluation of limulus amoebocyte lysate assay for diagnosis of, **207***
Gonorrhoea, screening in a prenatal clinic in South-east Asia, **134***
Gonorrhoea, single dose oral rosoxacin for, **347***
Gonorrhoea, three-day doxycycline treatment for, **135***
Gonorrhoea, treatment of, **68***
Gonorrhoea, treatment with cefotaxime, **208***
Gonorrhoea, treatment with co-trimoxazole, **347***
Gonorrhoea, treatment with rosoxacin, **279***
Granuloma inguinale, rare imported VD, **138***

Hepatitis A and B, increased risk in men with syphilis, **349**
Hepatitis A in homosexuals, **70***
Hepatitis B among homosexuals in Holland, **349***
Hepatitis B, asymptomatic rectal mucosal lesions and HBsAg at sites of sexual contact in homosexuals, **349**
Hepatitis B, epidemiology of sexually transmitted infections in heterosexuals, **349***
Hepatitis B, strategies for prevention of, as an STD, **349***
Hepatitis in male and female homosexuals, **349***
Herpes, genital, intravenous acyclovir for treatment of primary, **348***
Herpes, genital, risk of recurrence after first episodes, **136**
Herpes, genital, treatment of, **348***
Herpes simplex, chronic perianal in homosexuals: severe acquired immunodeficiency, **348***
Herpes simplex, genital, **136***
Herpes simplex, recurrent, systemic antiviral agents in, **70***
Herpes simplex virus and *C trachomatis* IgA in cervical secretions of patients with cervical atypia, **279***
Herpes simplex virus, management of genital infections during pregnancy, **280**
Herpes simplex virus, rapid technique for typing, **348***
Herpes simplex virus transformed and non-transformed cells, inhibition by acycloguanosine, **136***
Herpes simplex virus type 1, concurrent oral and genital infection by restriction endonuclease analysis, **136***
Herpes simplex virus type 1, genital infection with, **348***

Herpes simplex virus types 1 and 2, growth in fertile hens' eggs, 70*
Herpes simplex virus types 1 and 2, inactivated, induction of uterine cancer, 209*
Herpes virus-induced antigens in squamous cell carcinoma-in-situ of vulva, 70
HLA-B5 association with Behçet's disease in Mexican Mestizo population, 70*
Holland, hepatitis B among homosexuals in, 349*
Homosexual report on STD, 137*
Homosexuals, aetiology of anorectal infections, 209
Homosexuals, amyl nitrite alters T lymphocytes?, 349
Homosexuals, asymptomatic rectal mucosal lesions and HBsAg at sites of sexual contact in, 349
Homosexuals, attitude to VD clinics, 280*
Homosexuals, chronic perianal herpes simplex: severe acquired immunodeficiency, 348*
Homosexuals, female, failure to identify VD in, 137
Homosexuals, hepatitis A in, 70*
Homosexuals, hepatitis B in Holland, 349*
Homosexuals, male and female, hepatitis and other STD in, 349*
Homosexuals, *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy, 348
Homosexuals, serotypes of *N meningitidis* in, 346
Human papilloma virus type 1 from genital warts, 137

Imidazoles, *Candida* spp. sensitivity to, 136*
Italy, chlamydial infections in, 348*
Italy, epidemiology and aetiology of non-gonococcal urethritis in, 69*

Lactoferron, ability of *N meningitidis, N gonorrhoeae,* and commensal *Neisseria* to obtain iron from, 347*
Lesbians, failure to identify VD in, 137
Limulus amoebocyte lysate assay for presumptive diagnosis of gonorrhoea, 207*
Limulus lysate test kit, rapid presumptive diagnosis of gonococcal urethritis, 347*
Limulus test device for rapid evaluation of gonococcal and non-gonococcal urethritis, 207

McCoy cells, treated with emetine, growth of *Chlamydia* in, 348*
Meningococci, presumed sexual transmission of, 280
Metronidazole metabolism in cultures of *T vaginalis* and *E histolytica,* 209
Minocycline as treatment for non-gonococcal urethritis, 135*
Molluscum contagiosum, 210*
Mycoplasma, newly discovered in genital tract, 68
Mycoplasma, recovery and identification from human genital tract, 135*
Mycoplasmas in male genital tract infections, 68*
Mycoplasma hominis and *U urealyticum* in cervix and anal canal of women, 208*
M hominis, C trachomatis and *N gonorrhoeae* in patients with acute pelvic inflammatory disease, 208*

Neisseria commensal species, ability to obtain iron from lactoferron, 347*
Neisseria gonorrhoeae, ability to obtain iron from lactoferron, 347*
N gonorrhoeae, activity of MK-0366 against, 135*
N gonorrhoeae, ampicillin treatment, study in rabbits, 135*
N gonorrhoeae and *C trachomatis* prevalence in two different female populations, 68*
N gonorrhoeae and *N meningitidis,* monoclonal antibody analysis of lipopolysaccharide, 279*
N gonorrhoeae, antibiotic susceptibility in relation to serogroups, 207*
N gonorrhoeae, antigen-specific serotyping on outer membrane protein, 68
N gonorrhoeae, auxotypes and antibiotic susceptibilities in women with acute complicated and uncomplicated genital infection, 135*
N gonorrhoeae, β-lactamase plasmid in, characterisation of, 68*
N gonorrhoeae, β-lactam plasmids, retention of Tn-3-like transposon, 279*
N gonorrhoeae, comparative activity of cefemoxine, cefoperazone, cefuroxime, cefoxitin, and penicillin against, 279*
N gonorrhoeae, determinants of survival with human phagocytes, 347*
N gonorrhoeae, disc agar diffusion antibiotic susceptibility testing, 207*
N gonorrhoeae, effect of analgesics and lubricants on isolation of, 347*
N gonorrhoeae, effect of menstrual cycle and contraception on the recovery of, 346
N gonorrhoeae, effect of outer membrane antibody on virulence, 279*
N gonorrhoeae, evaluation of Phadebact test for identification of, 135
N gonorrhoeae from pelvic inflammatory disease, β-lactam susceptibility, 135*
N gonorrhoeae, human erythrocyte, receptors for, 207*
N gonorrhoeae, identification by standard and experimental tests, 347*
N gonorrhoeae, identification of new genetic site that effects sensitivity to normal human serum, 347*
N gonorrhoeae, induction of resistance to human serum by factors in human serum, 347*
N gonorrhoeae, isolation on haemoglobin-free NYC medium, 135*
N gonorrhoeae lipopolysaccharide, host species-specific damage to oviduct mucosa, 279*
N gonorrhoeae, mobilisation of resistance plasmids by 24·5 megadalton plasmid of, 208*
N gonorrhoeae, M hominis, and *C trachomatis* in patients with acute pelvic inflammatory disease, 208*
N gonorrhoeae outer membrane protein 1 is a porin, 279*
N gonorrhoeae outer membrane proteins, role in survival of, 279*
N gonorrhoeae P9, identification of novel pilus types, 68*
N gonorrhoeae, pathogenic mechanisms on human fallopian tubes by type 1 or type 4 colonies, 67*
N gonorrhoeae, peptidoglycan synthesis, effects of β-lactam antibiotics on, 135*
N gonorrhoeae, penicillinase-producing emergence of new plasmid combination in Britain, 347
N gonorrhoeae, penicillinase-producing from Canada, molecular and phenotypic characterisation of, 67*
N gonorrhoeae, penicillinase-producing, in Canada, 210*

N gonorrhoeae, penicillinase-producing, in Britain, 137*
N gonorrhoeae, penicillinase-producing in USA, 1976-1980, 210*
N gonorrhoeae, penicillinase-producing, serotyping and auxotyping in Sweden, 137*
N gonorrhoeae, Phadebact test for identification of, 347*
N gonorrhoeae, pharyngeal carriage, 207*
N gonorrhoeae, production of antibodies against a strain-specific cell surface antigen, 68*
N gonorrhoeae, proteolytic enzymes on outer membrane proteins of, 68*
N gonorrhoeae, relation of protein 1 and colony opacity to serum killing of, 347*
N gonorrhoeae, sensitivity to antibiotics by dilution and diffusion methods using a new medium, 207*
N gonorrhoeae, serological classification by coagglutination, 208*
N gonorrhoeae serology: detection of protein 1 antigens, 279*
N gonorrhoeae serotype protein agglutinogens, 279*
N gonorrhoeae, spectrum of infection with, 347*
N gonorrhoeae strains, cephalosporins activity against, 135*
N gonorrhoeae, superoxol (catalase) test for identification of, 347*
N gonorrhoeae, toxicity of lipopolysaccharide on human fallopian tube mucosa, 67*
N gonorrhoeae, toxicity on human fallopian tube mucosa, 67*
N gonorrhoeae, transport, evaluation of Stuart's medium in culturettes, 347*
N meningitidis, ability to obtain iron from lactoferron, 347*
N meningitidis and *N gonorrhoeae,* monoclonal antibody analysis of lipopolysaccharide, 279*
N meningitidis, serotypes in homosexuals, 346
N meningitidis strains, cephalosporins activity against, 135*
Neisseria species, evaluation of New York City medium to differentiate, 347*
Neurosyphilis, 134*
Neurosyphilis in Denmark, 277
Neurosyphilis, intrathecal synthesis of oligoclonal antibodies to *T pallidum,* 346*
New York City medium, evaluation of differentiation of species of *Neisseria,* 347*
Non-gonococcal and gonococcal urethritis, application of limulus test device in rapid evaluation of, 207
Non-gonococcal urethritis, comparison of Gram-stained urethral smear and first-voided urine sediment in diagnosis, 348*
Non-gonococcal urethritis, diagnosis of, 68*
Non-gonococcal urethritis, doxycycline treatment for, 135*
Non-gonococcal urethritis, epidemiology and aetiology in Italy, 69*
Non-gonococcal urethritis, minocycline as treatment for, 135*
Non-gonococcal urethritis, review of epidemiology and control, 135*
Non-gonococcal urethritis, semi-quantitation of urethral leucocytes in, 348*

Optic neuritis, acute syphilitic, 134*

Pelvic inflammatory disease, aetiology, diagnosis, and treatment of, 349*
Pelvic inflammatory disease, diagnosis and treatment in emergency room, 138*
Pelvic inflammatory disease in USA, epidemiology and trends in hospitalised women, 138*
Pelvic inflammatory disease in USA, incidence and trends in private practice, 138*
Penicillin, activity against *N gonorrhoeae,* 279*
Penicillin G *vs* piperacillin treatments for gonoccal urethritis, 279*
Penicillin, intravenous high dosage *vs* carbamazepine therapy for tabetic lightning pains, 206*
Penicillin levels in CSF during treatment of latent syphilis, 277
Penicillin-treated cases of acute syphilis in 1943-50: a survey, 277*
Penicillin, treponemacidal level in CSF, 134*
Penis, median raphe, infection with *T vaginalis,* 279*
Phadebact gonococcus test, evaluation of, 135
Phadebact test for identification of *N gonorrhoeae,* 347*
Piperacillin, lack of activity against *C trachomatis,* 348*
Piperacillin *vs* penicillin G treatments for gonococcal urethritis, 279*
Pneumocystis associated with infant preumonitis, 210
Pneumocystis carinii pneumonia in previously healthy homosexuals, 348
Pneumonitis, infant, associated with cytomegalovirus, chlamydia, pneumocystis and ureaplasma, 210*
Podophyllotoxin, ethanolic, self-treatment of penile warts with, 210*
Pregnancy, management of herpes simplex virus genital infections during, 280
Progesterone in mouse model for infection with *C trachomatis,* 279*
Prostitutes, STD among, 280*

Reiter's disease, 279*
Reiter's syndrome associated with *Campylobacter foetus* spp *jejuni,* 136*
Reiter's syndrome, cell-mediated immune responses to synovial mononuclear cells to sexually transmitted, enteric and mumps antigens, 136*
Reiter's syndrome, evaluation of criteria, 69, 136
Reiter's syndrome, review and case report, 136*
Rosoxacin in treatment of gonorrhoea, 279*
Rosoxacin, single oral dose for gonorrhoea, 347*

Sacroiliitis in Behçet's disease, 280*
Salpingitis, acute, *C trachomatis* isolated from fallopian tubes, 208
Salpingitis, acute, infection with *M hominis, C trachomatis,* and *N gonorrhoeae,* 208*
Sclerosing lymphangitis of penis, chlamydial aetiology? 208*
Sexual offenders, STD among, 280*

Spectinomycin and gentamicin compared in penicillin-resistant gonococci, **135***
Spirochaetes, oral, helical conformation of, **67**
STD among prostitutes and other sexual offenders, **280***
STD and cervical atypia, **349***
STD, initiation of prevention/training programme, **137***
STD in male and female homosexuals, **349***
STD statistics, **349***
STD, therapeutic decisions in the treatment of, **138***
Stuart's medium in culturettes, evaluation of *N gonorrhoeae* transport, **347***
Sweden, serotyping and auxotyping of β-lactamase *N gonorrhoeae,* **137***
Syphilis, acute optic neuritis, **134***
Syphilis, acute, survey of penicillin-treated cases in 1943-50, **277***
Syphilis and gonorrhoea, epidemiology update, **137***
Syphilis, current features of clinical picture and course, **134***
Syphilis, diagnosis of, **67***, **277***
Syphilis, early experimental, effect of cortisone administration on host-parasite relationships in, **206**
Syphilis, early, intensive arsenotherapy of, **345***
Syphilis, experimental, accelerated lesion development in, **278***
Syphilitic immune cells, transfer of resistance: lack of correlation with mitogenic activity, **278**
Syphilis, increased risk of hepatitis A and B in men with, **349**
Syphilis, latent, CSF penicillin levels during treatment of, **277**
Syphilis, monorecidive chancre, **206***
Syphilis, outcome of treated among Indian armed forces personnel, **345**
Syphilis, phlebitis simulating branch vein occlusions, **134***
Syphilis, primary of tonsils, **134***, **345***
Syphilis, secondary, in-situ identification of mononuclear cells in cutaneous infiltrates in discoid lupus erythematosus, sarcoidosis and in, **206***
Syphilis, secondary with uveitis, **134**
Syphilis, serodiagnosis, **206***
Syphilis serology, FTA-ABS double-staining procedure, **134***
Syphilis serology, heated versus unheated sera in TPHA test, **345***
Syphilis serology—routine screening in hospital, **278***
Syphilis serology, screening test added to congenital hypothyroidism screening, **345***
Syphilis serology; specificity, sensitivity and reproducibility among FTA-ABS, TPHA, and micro-TPHA tests, **206***
Syphilis serology, unheated reagin test, **345***
Syphilis, treatment of, **345***
Syphilitic alopecia, **345***

Tabes dorsalis, lightning pains, intravenous penicillin *vs* carbamazepine therapy, **206***
Tetracycline, one-week treatment in chlamydial urethritis, **69***
Tinidazole in treatment of partners of females with trichomoniasis, **70***
Tonsil, syphilitic primary on, **134***, **345***
TPHA test, heated versus unheated sera, **345***
Treponema, characterisation of strain isolated from a pleural effusion, **346***
Treponema denticola, helical conformation of, **67**
Treponema Reiter, isolation of flagella from, **346***
Treponema Reiter, isolation of heat stable antigen using an immunoadsorbent with antibodies from syphilitic patients, **206***
Treponema Reiter RNA antigen, purification by hydrophobic interaction chromatography, **278***
T pallidum, avoidance of host defences by, **278**
T pallidum, cultivation of virulent, in tissue culture, **67**
T pallidum, helical conformation of, **67**
T pallidum, loss during incubation in Nelson's survival medium, **346***
T pallidum, microaerophilic nature of, **346***
T pallidum, oligoclonal antibodies, intrathecal synthesis in neurosyphilis, **346***
T pallidum, pathogenesis and immunology of, **206***
T pallidum, plasmid DNA in, potential for antibiotic resistance by syphilis bacteria, **134**
T pallidum, purification of related Reiter treponemal protein antigen, **346***
T pallidum, replication in tissue cultures of Sf1Ep cells, **346**
T pallidum, rectal mass caused by, confirmation by immunofluorescent staining, **277**
T paraluis-cuniculi, helical conformation of, **67**
Trichomonas vaginalis, activitation of alternate complement pathway, **209***
T vaginalis and *E histolytica,* metronidazole metabolism in cultures of, **209**
T vaginalis, evaluation of acridine orange stain for, **279***
T vaginalis infection in median raphe of penis, **279***
T vaginalis, sensitivity to zinc, **209***
Trichomoniasis, tinidazole in treatment of partners of females with, **70***

Ulcers, genital, characterisation of an unusual bacterium isolated from, **350**
Ureaplasma associated with infant pneumonitis, **210***
Ureaplasma urealyticum and *M hominis* in cervix and anal canal of women, **208***
U urealyticum, incidence in women attending STD clinic, **208***
Urethral syndrome, isolation of *C trachomatis* from, **208***
USA, pelvic inflammatory disease in, epidemiology and trends in hospitalised women, **138***
USA, pelvic inflammatory disease in, incidence and trends in private practice, **138***
USA, penicillinase-producing *N gonorrhoeae* in, 1976-1980, **210***
Uveitis, secondary syphilis with, **134**

Vaginal bacteria in normal young women, **70**
Vaginal tampons, recovery of *C trachomatis* and cytomegalovirus from, **348***
VD clinics, attitude of homosexuals to, **280***
VD, history of development of service for, **350***

Vulva, squamous cell carcinoma-in-situ, herpes virus-induced antigens in, **70**
Vulvovaginitis, current therapy of, **349***

Warts, anal, malignant transformation, **210***
Warts, genital, **349***
Warts, genital, human papilloma virus type 1 from, **137**
Warts, genital, management with carbon dioxide laser, **280**, **280***
Warts, penile, self-treatment with ethanolic podophyllotoxin for three days, **210***

Yeasts other than *C albicans* in vagina of normal women, **70***

Zinc, sensitivity of *T vaginalis* to, **209**

Br J Vener Dis 1982;**58**:424-7

Index to authors of articles abstracted, 1982

**Indicates that only the title is given.*

Adams H G, 136
Adcock M, 207* *(bis)*
Adler M W, 348*, 349*, 350*
Ahrons S, 208* *(bis)*, 348*
Alarçon-Segovia, 70*
Al-Egaily S, 134*
Alexander E R, 68*
Alexander R, 135*
Anand S, 345
Annat D J, 70
Apicella M A, 279*
Arko R J, 347*
Arnett F C, 69, 136
Arya O P, 136
Ashton F E, 347*
Asnani P J, 68*
Axelsen N H, 206* *(bis)*, 278*, 346* *(bis)*

Babayants R S, 134*
Back A, 210*
Bailey J, 209*
Bain S S R, 208*
Baker-Zander S A, 206
Ballard R C, 350
Baltch A C, 134
Barrett D, 137*
Barron A L, 208*
Beaulieu B B, 209
Belin M W, 134
Bennet B D, 209*
Bennett K M, 279*
Bentzon M W, 346*
Biagini G, 346*
Biddle J W, 210*, 347*
Bigelli A J, 136*
Bitter T, 69, 136
Bjerke J R, 206*
Blackman E, 347*
Blake M S, 68*
Blount J H, 138* *(bis)*
Blundell J K, 135*
Boehm F H, 280
Bond W W, 349
Borrie-Hulme C J, 68*, 348*
Boslego J W, 207*
Bouvier F, 68*
Bowie W R, 68*, 135*, 208*, 348* *(bis)*
Brasfield D M, 210*
Bredt W, 68*
Brodeur B R, 347*
Brooks G F, 347*
Brown M B, 210*
Brown S T, 210*, 347
Bruce A W, 279*
Brunham R C, 208*
Buchanan T B, 68, 279*
Burek J, 70
Burgener L, 208*
Bustamente R, 137
Bygdeman S, 137*, 207*, 208*

Caliando J J, 135*
Calkins J W, 280
Cannon J G, 7*, 68*
Cantini A, 345*
Capen C V, 280
Carlson B L, 347*
Carpenter J L, 210*
Casin I, 349*
Cevenini R, 69*, 348*
Chadwick P, 279*
Chaffee R F, 209
Chambon J, 207*
Chan J K, 278
Chapel T A, 207*
Cheri Lloyd R M, 206
Chowdhury M N H, 135*
Christensen J, 70*
Christiansen R O, 347*
Christiansen S, 346*
Clecner B, 347*
Cleveland P H, 348*
Cole R M, 68
Collins B N, 280
Conrad G L, 280*
Cooke E M, 135
Corey L, 136, 209
Costerton J W, 280*
Courvalin P, 207*
Coutinho R A, 349*
Cover W H, 346*
Cox D L, 67, 346
Crumpacker C S, 136*
Curson P M, 278*

Danielsson D, 137*, 208*
Da Rosa D M, 136*
Darrow W W, 137*, 280*
Davidson R L, 136*
Dawson C R, 135*
Delbeke E, 207*
Del Guidice-Knipping J A, 70*
Desai K, 348*
Dickgiesser N, 137*
Diena B B, 347*
Dillon J A R, 67*
Dillon J R, 210*
Domer J E, 280*
Douglas J T, 279*
Douglas L J, 280*
Draper D L, 135*
Dreesman G R, 70
Drexler L, 347*
Dube J-L, 70*
Ducas J, 277
Duck P, 67*
Dudley D K L, 136*
Dunlop E M C, 134*
Duposoir J L, 135*

Ebright J E, 347*
Ellis R E, 136*
Elmros T, 135*
Embil J A, 136*
Eschenbach D A, 348
Estes W, 280
Evans Z A, 136*

Falcieri E, 346*
Fayet D, 279*
Feldman D, 209*
Fenger C, 135*
Fiddian A P, 348*
Field H J, 70*
Fieldsteel A H, 67, 346
Finlay-Price K J, 347*
Fisk P, 279*
Fitzgerald T J, 206*, 278*
Fitzpatrick J E, 210
Fiumara N J, 135*, 345*, 347*
Flett F, 208*
Floyd J F, 135*
Folk J C, 134*
Ford D K, 136*
Fortier M, 70*
Fraga A, 70*
Frasch C E, 346*
Fraser J, 277*
Froment Y, 279*
Furr P M, 135*, 208*

Gandiol C, 349*
Gardner W A, 209*
Gasponi A, 346
Gaudenz R, 208*
Gehle D, 210*
Geow Y F N, 134*
Gettner S M M, 136*
Gillin F D, 209*

Gimenéz-Roldán S, 206*
Glynn A A, 207*
Goedert J J, 349
Goei S H, 138
Goh R H, 134*
Goldacre M J, 70
Goldacre J, 70*
Goldman J A, 279*
Goldman P, 209
Goodell S, 277
Gotschlich E C, 68*
Gottlieb M S, 348
Grady G F, 349*
Gramstad N D, 210
Granato P A, 135*
Greenfield R A, 280*
Greenwood J R, 279*
Gregg C R, 67* *(bis)*, 279*
Growdon J F, 280
Gruninger R P, 135*, 279*
Guibourdenche M, 207*
Guymon L F, 347*
Gyves M T, 68*

Hadfield S G, 207*
Hadley W K, 135*
Hagedorn H-J, 67*
Hahn G A, 280*
Halder H R, 345
Haley M S, 347*
Hamble E A, 206*, 345*
Hammer G S, 348*
Handley J O, 135*
Handsfield H H, 137*, 208*, 279* *(bis)*
Harris R I, 70
Hart D J, 136*
Hay P B, 134
Heckels J E, 68*, 279*
Hector R F, 280*
Heggie A D, 68*, 209*
Heite H-J, 67*, 68*
Hellerqvist G G, 67*
Hermerath C A, 279*
Hildebrandt J F, 68
Ho P, 134*
Hobson D, 69*
Holm S E, 135*
Holmes K K, 67*, 135*, 137*, 208*, 279* *(bis)*
Honkonen E, 279*
Houang E T, 134*
Houston J G, 280*
Hoyer H, 138*
Humphreys G O, 208*
Hunter E, 134*

Ison C A, 207*

Jaffe H W, 210*
James J F, 135*, 347*
Jay K, 137*
Jephcott A E, 137*
Jessamine A G, 210*
Johanisson G, 69, 208*
Johnson A P, 67*, 279* *(bis)*, 347*
Johnson R C, 67
Johnson S R, 210*
Johnston N A, 347
Jones J M, 280*
Jones O, 138* *(bis)*
Jørgensen B B, 277
Judson F N, 135*, 279*, 349*, 349
Juvakoski T, 69*

Kahn S, 347*
Kaklamani E, 137*
Kalimo K, 279*
Kallings I, 137*
Kampmeier R H, 277*, 345*
Kanerva L, 69*
Kapur T R, 345
Karayiannis P, 69*
Kaufman R H, 70
Kaufmann E R, 136*
Kay F, 135*
Kelly J R, 347*
Kennedy E J, 134*
Khan M Y, 135*, 279*
Kirk-Hillaire K, 279*
Kjellberg E, 135*
Kleris G S, 280*
Knapp J S, 279*
Koch R L, 209
Koester C M, 68*
Kolator B, 347
Korhonen O M, 70
Korting H C, 207*
Kosunen T U, 136*
Koutsky L, 348
Kraus S J, 210*, 348*
Kristensen J K, 208*
Krogh H-K, 206*
Kryger P, 349
Kumar B, 68*
Kumar T, 68*
Kuo C-C, 208*, 348*
Kuziez M N, 210
Kwaasi A A A, 347*

Lambden P R, 68*, 279*
Lammel C J, 347*
Landis S J, 279*
Laplack M, 348*
Larew M S, 348*
Larsen S A, 206*, 345* *(bis)*
Lassus A, 69*
Laudat P, 135*
Lauharanta J, 69*
Laurin J, 348*
Lavalle C, 70*
Lee M D, 279*
Lee N, 69*
Lee S H, 210
Lefevre J C, 68*
LeFrock J L, 278
Lexow S S, 134*
Lian C J, 207*
Limson B M, 347*
Lobes L A, 134*
Loose D L, 209*
Lopez C, 348*
Loudon N, 70* *(bis)*
Lue Y A, 347*
Luger A, 277*
Lukehart S A, 206, 277
Lumicae G G, 68*
Lycke E, 208*
Lyng J, 70
Lyon F E, 280*

Macaset R K, 347*
McBride H M, 68, 279*
McClean A N, 137*
McCormack W M, 346, 347*
McCourtie J, 280*
McFarlane E S, 136*
McGee Z A, 67* *(bis)*
McGregor D H, 210
McKenzie D W R, 136*
McLafferty J R M A, 209
McNeil P J, 135*
McNicol P, 207*
Maeland J A, 279*
Magrina J F, 280
Mallinson H, 136
Malouf M, 70*
Mansheim B J, 279*
Manuel F R, 136*
Manzon L M, 68*, 348*
Mårdh P-A, 135*, 208* *(bis)*, 348*
Margolis S, 137*
Marrie T J, 280*
Martin D H, 208*, 348
Martin M, 206*
Martin P M V, 347*
Martio J, 136*
Masr M S, 348*
Masterson B J, 280
Mathieson L, 349
Matre R, 206* *(bis)*
Matson D O, 70
Mehta S P, 206*
Melly M A, 67* *(bis)*
Mendelson J, 347*
Michaelsen T E, 346*
Michel V P, 209
Mickelsen P A, 347*
Mildvan D, 137
Miller J N, 134, 346*
Milne L J R, 70*
Mindel A, 70, 348*
Mittler R S, 68*
Moeckli R A, 67, 346
Møller B R, 135*, 208*, 348*
Morel P, 349*

Morin G, 70*
Müller F, 206*
Munday P E, 208*
Murphy V L, 279*
Myers M G, 348*

Nachamkin I, 68*
Nachum R, 347*
Nalley J F, 210*
Nehaul B B G, 135
Nelson J D, 208
Neuland C Y, 349
Neumann H H, 135* *(bis)*
Niehaus C, 345*
Nielsen J O, 349
Nikaido H, 279*
Nilsson S, 208*
Norgard M V, 134
Norris S J, 346*
Nowinski R C, 348*
Nüssler E, 208*

Oberhoffer T, 210*
O'Hara C E, 209*
Oppenheimer E H, 346
Oriel J D, 349*
Osborn M F, 347*
O'Shaugnessy M, 279*
Oxman M N, 348*

Paavonen J, 135*
Panconesi E, 345*
Pareek S S, 135*, 345*
Parsons N J, 347* *(bis)*
Patamasucon P, 208
Patel P V, 347*
Paul I D, 348*
Pauze M, 210*
Pederson N S, 206* *(bis)*, 134*, 346* *(bis)*, 349
Pender B J, 134*
Penn C W, 278
Perdrup A, 277
Perkins H R, 135*
Perryman M W, 345*
Petersen C S, 206*, 278*, 346* *(bis)*
Petersen N S, 278*
Pettit D E, 206*, 345*
Peutherer J F, 348*
Philips S C, 137
Piffaretti J C, 279*
Pindah S, 279*
Pinon G, 135*
Podgore J K, 67*
Ponka A, 136*
Pope V, 345*
Portnoy J, 347*
Powell K R, 135*
Prere M F, 68*
Pretzer E, 207*
Primrose D S, 207*
Prior R B, 207

Quentin R, 135*
Quinn R W, 277*
Quinn T C, 209, 277

Ramphal R, 279*
Rank R G, 208*
Rao K N A, 209*
Reagan J W, 209*
Reeves W C, 136
Rein M F, 138*, 209, 349*
Reiner N E, 349
Rest R F, 207*
Rettig P J, 208
Reynolds G H, 346
Richman D D, 348*
Rides A, 136*
Riou J Y, 207*
Roberts D E, 279*
Robertson D H H, 348*
Robertson P, 137
Robson H G, 277, 348*
Rodewald R R, 135*
Rodgers F D, 70*
Rodriguez V L, 279*
Røe S F, 279*
Rose D L, 68
Ross M W, 280*
Rumpianesi F, 69*
Rush B, 280*

Sadoff J C, 207*
Saginur R, 347*
Sakell E, 136*
Salari S H, 68
Salit I E, 346
Sallou P, 135*
Salomonsky N L, 135*
Sander J, 345*
Sandström E, 208*
Sandström E G, 279*
Sarvamangala J N, 209*
Saunders J, 208*
Schachter J, 135*, 137, 349*
Schanker H M, 348
Scheibel J, 208*
Schell R F, 278
Schneible Smith C, 135*
Schroff R, 348
Schultz B, 208*
Schurman D J, 209*
Schut B J T, 349*
Scnipper L E, 136*
Sell S, 206
Shafer W M, 347*
Shah P, 136*
Sher A, 209*
Shivananda P G, 209*
Sicard D, 207*
Siddiqui Y, 279*
Siegal F P, 348*
Sikotra S, 136
Simms D H, 347*
Simpson M L, 279*
Singha H K S, 348*
Smith B, 207*
Smith C A F, 135*
Smith H, 347*
Smith I W, 348*
Smith K E, 347*
Socoff S L, 208*
Soendjojo A, 279*
Spagna V A, 207
Sparling P F, 347* *(bis)*
Speckards A, 134*
Stagno S, 210*
Stamm R, 136*
Stamm W E, 68*, 137*
Staquet M J, 137
Stepan D E, 67
Stephens R S, 348
Stimson J B, 135*
St John R K, 138* *(bis)*
Stuart L A, 68*
Subramanian S, 134*
Sutherland S, 348*
Swanson J, 68*
Sweeney A, 277*
Sweet R L, 135*, 138*, 349*
Swensson L, 208

Talbot M D, 280
Tam M R, 348*
Taylor-Robinson D, 67*, 68, 135*, 208* *(bis)*, 279* *(bis)*
Tedder R, 70*
Teoh S K, 134*
Terho P, 279*
Thabaut A, 135*
Thivolet J, 137
Thomas D Y, 67*
Thompson S E, 347*
Tice A W, 279*
Tomkins D S, 135
Tozzi M A, 136*
Tramont E C, 207*
Trichopoulos D, 137*
Tuach S, 208*
Tuffrey M, 279*
Tully J G, 68
Tummon I S, 136*
Turner J A, 347*
Tuzlaci M, 280*
Tyler H, 210

Ursi J-P, 350

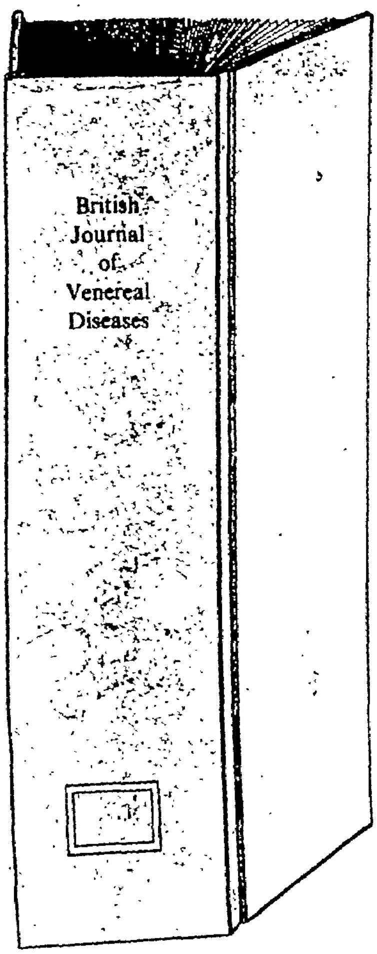
Vandvik B, 346*
Van Dyck E, 350
Van Lent N A, 349*
Van Slyke K K, 209
Varghese M N, 278*
Vargues R, 135*
Varotti C, 69*
Vartdal F, 346*

Vessey M P, **70** *(bis)*
Viac J, **137**
Viers W A, **134***
Virji M, **279***
Von Krogh G, **210**
Vontver L A, **136**

Walker E A, **345***
Wallen W C, **349**
Walters J H, **136***
Ward M E, **68**
Watt B, **70*** *(bis)*
Watt P J, **69***, **279***
Weil A, **208***
Weiner L B, **135***
Weinstein J M, **134***
Weismann K, **138***
Wells J I, **138**
Wentz W B, **209***
Weström L, **208**
White H J, **208***
Wiesner P J, **210***
Wildy D, **70***
Willett W S, **279***
William D C, **137**, **349***
Willkens R F, **69***, **136**
Winblad B, **135***
Winn K J, **346**
Wiseman G M, **207***
Wong K-H, **347***
Worthen D M, **348***
Wright P F, **280**

Yazici H, **280***
Yoder B L, **68***
Young A, **137***, **208***
Young H, **208***
Yurkakul S, **280***

Zaidi A A, **138***
Zuccati G, **345***
Zudin B I, **134***
Zurlinden E, **347***



'CORDEX'

SELF-BINDING CASES

for easy and instant reference

Made to hold one volume (6 issues) of your **BRITISH JOURNAL OF VENEREAL DISEASES.** Each case is handsomely bound with a colour to complement the journal. Spring nylon cords, to carry the journals, slide freely along the spine of the binder. The name of the journal is gold-blocked on the spine. Date labels are also provided.

Price: Inland £4.00; Overseas US$12.00
including postage (air mail overseas) and packing

ORDER NOW

From: The Subscription Manager,
BRITISH JOURNAL OF VENEREAL DISEASES
BMA House, Tavistock Square, London WC1H 9JR

British Journal of Venereal Diseases December 1982 Vol 58 No 6

A journal of the sexually transmitted diseases and allied disorders edited for the Medical Society for the Study of Venereal Diseases

Contents

Treponemal serology on Bali Island, Indonesia
R NEY, M F GARNER, J L BACKHOUSE, N WIRIA DUARSA, DARWATA BREGUET, AND G BREGUET *page* 351

Syphilis in pregnant women in Zambia
A V RATNAM, S N DIN, S K HIRA, G J BHAT, D S O WACHA, A RUKMINI, AND R C MULENGA *page* 355

Detection of *Neisseria gonorrhoeae* antigen by a solid-phase enzyme immunoassay
H A AARDOOM, D DE HOOP, C O A ISERIEF, M F MICHEL, AND E STOLZ *page* 359

Induction in gonococci of phenotypic resistance to killing by human serum by human genital secretions
P M V MARTIN, P V PATEL, N J PARSONS, AND H SMITH *page* 363

Chlamydial genital infection in Ibadan, Nigeria: a seroepidemiological survey
S DAROUGAR, T FORSEY, A O OSOBA, R J DINES, B ADELUSI, AND G O COKER *page* 366

Chlamydial genital infection in Addis Ababa, Ethiopia: a seroepidemiological survey
T FORSEY, S DAROUGAR, R J DINES, D J M WRIGHT, AND P S FRIEDMANN *page* 370

Chlamydial urethral infection in Teheran: a study of male patients attending an STD clinic
S DAROUGAR, BARRIE R JONES, L CORNELL, J D TREHARNE, R ST C DWYER, AND B ARAMESH *page* 374

Pathogenic microbial flora of genital ulcers in Sheffield with particular reference to herpes simplex virus and *H ducreyi*
G R KINGHORN, S HAFIZ, AND M C McENTEGART *page* 377

Preparation and immunogenicity of vaccine Ac NFU$_1$ (S$^-$) MRC towards the prevention of herpes genitalis
G R B SKINNER, C B J WOODMAN, C E HARTLEY, A BUCHAN, A FULLER, J DURHAM, M SYNNOTT, J C CLAY, J MELLING, C WIBLIN, AND J WILKINS *page* 381

Clinic diagnosis of anaerobic vaginosis (non-specific vaginitis): a practical guide
A BLACKWELL AND D BARLOW *page* 387

Metronidazole resistance of *Trichomonas vaginalis* as a cause of treatment failure in trichomoniasis: a case report
JAROSLAV KULDA, MILENA VOJTĚCHOVSKÁ, JAN TACHEZY, PAVOL DEMEŠ, AND EVA KUNZOVÁ *page* 394

Scissor excision of anogenital warts
JOHN M GOLLOCK, KENNETH SLATFORD, JENNIFER M HUNTER *page* 400

Screening for syphilis among homosexual men in bars and saunas in Amsterdam
L H LUMEY, JEANNETTE KOK, AND R A COUTINHO *page* 402

Ulcerative vulvitis in Reiter's syndrome: a case report
S O'N DAUNT, K E KOTOWSKI, A P O'REILLY, AND A T RICHARDSON *page* 405

Correspondence *page* 408

Book reviews *page* 410

Notice *page* 410

Index *page* 411

ASTM CODEN: BJVDAK 58 (6) 351-427 (1982)

PUBLISHED BY BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON WC1H 9JR, AND
PRINTED IN ENGLAND BY DAWSON & GOODALL LTD., THE MENDIP PRESS, BATH

ISSN 0007-13